# Crop-weed interactions determined by sensor techniques

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# CROP-WEED INTERACTIONS DETERMINED BY SENSOR TECHNIQUES


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# Preface

This dissertation is submitted together with four enclosed papers in partial fulfilment of the requirement of the Philosophiae Doctor (Ph.D.) degree at the University of Copenhagen, Faculty of Life Sciences, Denmark.

The work presented here has been carried out at the University of Aarhus, Department of Integrated Pest Management, Research Centre Flakkebjerg as a part of the project "Characteristics of spring barley varieties for organic farming, BAR-OF". This project was granted by the Danish Agricultural Research Centre for Organic Farming (DARCOF-II-VI-2). The Research School for Organic Agriculture and Food Systems (SOAR) funded one year of studies, and the University of Aarhus, Department of Integrated Pest Management financed the rest.

*Supervisors*

- Associate Professor, Dr. Christian Andreasen, the University of Copenhagen, Faculty of Life Sciences
- Head of Research Unit, Dr. Niels Holst, the University of Aarhus, Faculty of Agricultural Sciences
- Senior Scientist, Dr. Henning Tangen Søgaard, the University of Aarhus, Faculty of Agricultural Sciences

*The Ph.D. study included*

1. Intensive experimental field work in the growing seasons 2003-2005 at the University of Aarhus, Faculty of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg.
2. Participation in following courses and conferences
    a. The SOAR summer school entitled "Values, Ideologies and Organic Agriculture" (4 ETCS)
    b. The SOAR summer school entitled "Globalisation: Threat or Opportunity for Organic Farming?" (4 ETCS)
    c. A Ph.D. course on Chemometrics and Multivariate Statistical Data Analysis (6 ETCS)
    d. A Ph.D. course on Mixed Linear Models (6 ETCS)
    e. A Ph.D course on Applied Statistics (6 ETCS)
    f. Two Ph.D. courses on Computer Vision (2+3 ETCS)

   g. A short course in the use of MatLab
   h. 13th European Weed Research Society Symposium, 19-23 June 2005, Bari, Italy
   i. Two autumn and one spring seminars arranged by SOAR
3. Participation in the project “Characteristics of Spring Barley Varieties for Organic Farming, BAR-OF” from application for funding to finalising the project.

# Foreword

Looking back on the years passed while working on this project; planning, conducting and analysing field trials, publishing and attending several very interesting end educational courses, it is time for making a kind of status on the progress that led to this result, which finally has materialised in the present thesis. Even though my name is on the cover and I am responsible for the content, I could not have finished it without the influence of

- my “staff” of supervisors, Christian, Niels and Henning. I provided you with both “whip and carrot” to be used freely on me, to keep me on the right track towards the goal. I owe you all a very special acknowledgement for the inspiring discussions, your patience when deadlines were postponed, helpful comments in the manuscripts and all the good advise through the project period,
- the good spirit and inspiring and sometimes hectic discussions in the forum of BAR-OF,
- the membership of SOAR, where I got the possibility of meeting Ph.D. students, working with other research areas of organic agriculture, and of discussing more or less philosophic aspects of organic agriculture,
- Karen Heinager, Eugene Driessen, Lena Christensen, Susanne Sindberg and Henrik Grøndal for all the hours you spent in the field trials collecting data. Without your great effort, it would never have been possible,
- Kristian Kristensen, your statistical help was inestimable,
- Ilse A. Rasmussen for good ideas and your commitment while I was conducting the experiment and in the writing phase,
- my colleagues at Research Centre Flakkebjerg, especially in Research Unit WEEDS for your interest in the project and help when it was needed,
- my wife Mette and my kids Astrid, Jeppe, Jens and Nils, who all have suffered from a husband or a father, that sometimes was in another world far from Stenkistevej. I am sure, I shall have returned, when you read this.

Flakkebjerg April 2008

Preben Klarskov Hansen

# Forord

Efter et tilbageblik på de forløbne år, hvor jeg har arbejdet på dette projekt; planlægning, gennemførelse og analyse af markforsøg, publicering, samt deltagelse i adskillige lærerige kurser, er tiden nu kommet, hvor der skal gøres status over forløbet, hvilket er udtrykt i nærværende afhandling. Selvom mit navn står på forsiden af afhandlingen og jeg er ansvarlig for indholdet, kunne jeg ikke have færdiggjort den uden indflydelse af

- min "vejlederstab," Christian, Niels og Henning. Jeg udstyrede jer både med "pisk og gulerod" som I frit kunne anvende på mig for at holde mig på sporet mod målet. Jeg skylder jer alle en særlig anerkendelse for de inspirerende diskussioner, jeres tålmodighed med udskudte deadlines, jeres hjælpsomme kommentarer til manuskripterne og alle de gode råd gennem projektperioden,
- den gode ånd og de inspirerende og til tider hektiske diskussioner i regi af BAR-OF,
- medlemskabet af SOAR, hvor jeg fik muligheden for at møde Ph.D.-studerende, som arbejder med andre forskningsområder inden for økologisk jordbrug og diskutere mere eller mindre filosofiske aspekter af økologisk jordbrug,
- Karen Heinager, Eugene Driessen, Lena Christensen, Susanne Sindberg og Henrik Grøndal: Tak for alle de mange timer I har brugt på dataindsamling i forsøgene. Uden jeres indsats ville det ikke have været muligt,
- Kristian Kristensen: din statistiske hjælp har været uvurderlig,
- Ilse A. Rasmussen: tak for gode ideer, samt dit engagement i forbindelse med udførelsen af forsøgene og i skrivefasen,
- alle medarbejderne på Forskningscenter Flakkebjerg, især i forskergruppen Ukrudtsøkologi og Beslutningsstøtte: Tak for jeres interesse i projektet og hjælp når det gjaldt,
- min kone Mette og mine børn Astrid, Jeppe, Jens og Nils, som alle har lidt under at have en mand eller far, som sommetider var i en anden verden langt væk fra Stenkistevej. Jeg er sikker på, at jeg er kommet tilbage når I læser dette.

Flakkebjerg april 2008

Preben Klarskov Hansen

# Contents

## Summary

Management of competition between crop and weeds is important to optimise yield, when the crop is grown in an organic or low-input system or when conventional farming wants to reduce the use of (or dependency on) herbicides to reduce the negative impact of these compounds on the environment. This Ph.D. thesis is concerned with the development of methods to measure the competition between crop and weeds in spring barley, and the interactions between a preventive and a direct weed control method. The main conclusions are mentioned below.

A study of 79 varieties of spring barley grown under both conventional and organic conditions showed that it was possible to use sensor based measurements of the crop growth conducted under conventional growing conditions to predict the cover of weeds under organic conditions. In this study we measured the weed suppressive ability of the varieties in two ways: 1) directly, by weed cover assessments under weedy conditions, and 2) indirectly, by sensor measurements of varietal growth traits (reflectance, leaf angle and culm length) under weed-free conditions. Based on the growth trait measurements, we successfully ranked the spring barley varieties according to how much they reduced the maximal observed weed cover (range between 12% and 55%).

In a two-year study of four spring barley varieties grown with and without competition with weeds at two different nutrient levels, we investigated the tolerance to weed harrowing of four pure varieties and examined the possible interactions between varietal weed suppressive ability and nutrient level. We defined tolerance as the combined effect of crop resistance (ability to resist soil covering) and crop recovery (the ability to recover in terms of yield). The weed harrowing strategy was a combination of one pre- and one post-emergence weed harrowing. In terms of yield, the four varieties responded significantly different to weed harrowing, and the response depended on nutrient level. At the lower nutrient level, weed harrowing caused an increase in yield of 4.4 hkg $ha^{-1}$ for a strong competitor (*cv.* Otira), while there was no effect on yield at the higher nutrient level. For a weaker competitor (*cv.* Brazil), weed harrowing caused no change in yield at the lower nutrient level, whereas yield decreased by 6.0 hkg $ha^{-1}$ at the higher nutrient level. There were marked differences between the weed suppressive ability of the four varieties when harrowing was omitted, with less pronounced but significant differences when harrowed. However, weed harrowing did not change the weed

suppressive ability of a variety. Further, we found a negative correlation between cover caused by harrowing and canopy height.

In the same field experiment as described above, we studied four scenarios of using sensor-based measurements of reflectance, vegetation cover and canopy structure (both for crop and weeds) in the early growth stages to estimate the potential yield of the crop. The results showed that by using 14 reflectance measurements conducted through the entire growing season, nine vegetation-cover measurements conducted until the presence of the second internodie in the elongation stage, as well as one measurement of weed density and two canopy structure measurements, an multivariate ordination technique using partial least squares (PLS) was able to explain 65% of the yield variation with seven principal components (PCs). By excluding weed density and canopy structure measurements, the predictability of the PLS model was not reduced. By using only the first five sensor-based measurements (before crop growth stage 21-22), the PLS model was able to explain 38% of the yield variation. Further reductions in the number of measurements reduced the accuracy of the model; however we found that a measurement 16-18 days after sowing alone explained 27% of the variation in yield. Compared to the variation explained by using all available measurements through the entire growing season, an early sensor-based measurement can give reasonable estimates of the expected yield helping the farmer to optimise the use of herbicides.

**Keywords:** Competition, crop-weed interactions, *Hordeum vulgare* L., image analysis, mechanical weed control, mixed linear models, multivariate statistics, reflectance, spring barley varieties, varietal differences.

# Sammendrag

Styring af konkurrencen mellem afgrøde og ukrudtet er en vigtig faktor, når man stiler efter et optimalt udbytte i økologiske eller lav-input dyrkningssystemer, eller hvis det konventionelle landbrug ønsker at reducere anvendelsen (eller afhængigheden) af herbicider for at reducere risici for negativ indflydelse på det omgivende miljø. Denne PhD-afhandling omhandler udviklingen af metoder til måling af konkurrencen mellem afgrøde og ukrudtet i vårbyg samt en undersøgelse af vekselvirkningerne mellem en præventiv og en direkte metode til ukrudtsbekæmpelse. Hovedkonklusionerne nævnes i det følgende.

En undersøgelse af 79 vårbygsorter dyrket under både konventionel og økologiske forhold viste, at det var muligt at anvende sensor-baserede målinger af sorternes vækst under konventionelle forhold til at prediktere dækningsgraden af ukrudt under økologiske forhold. I undersøgelsen målte vi sorternes evne til at undertrykke ukrudtet på to måder 1) direkte, ved bedømmelser af ukrudtsdækningen under ukrudtsfyldte forhold under økologiske forhold og 2) indirekte, ved sensormålinger af sorternes vækstkarakteristika (reflektans, bladvinkel og strålængde) under ukrudtsfrie forhold. Udfra de indirekte målinger, var vi i stand til at indeksere sorternes evne til at undertrykke ukrudt, som rangerede fra 12%-55% af den maksimale dækningsgrad.

I et to-årigt forsøg med sorter af vårbyg, dyrket med og uden konkurrence med ukrudt under to næringsstofniveauer, var målet at undersøge tolerancen overfor ukrudtsharvning af fire rene sorter samt at undersøge mulige vekselvirkninger mellem sortens evne til at undertrykke ukrudt og det givne næringsstofniveau. Tolerancen blev defineret som en kombineret effekt af afgrødens evne til at modstå jordtildækning og afgrødens efterfølgende genvækstevne. Ukrudtsharvningen blev gennemført som kombination af en blindharvning og en almindelig ukrudtsharvning. Hvad angår udbyttet, reagerede sorterne forskelligt på ukrudtsharvning og denne respons var afhængig af det pågældende næringsstofniveau. I det lave næringsstofniveau forårsagede ukrudtsharvning en udbyttestigning på 4.4 hkg $ha^{-1}$ i den konkurrencestærke sort Otira, mens der ikke var nogen signifikant effekt i det høje næringsstofniveau. I den noget svagere konkurrent Brazil forårsagede ukrudtsharvning ingen signifikant udbytteændring ved det lave næringsstofniveau, mens udbyttet blev reduceret med 6.0 hkg $ha^{-1}$ i det høje næringsstofniveau. Der var markante sortsforskelle, hvad angår deres evne til at undertrykke ukrudtet, når de ikke blev harvet, og denne evne var der stadig men mindre udtalt, når der blev harvet. Der var dog ingen vekselvirkning mellem sortens evne til at undertrykke ukrudt og ukrudtsharvning. Vi fandt endvidere en sammenhæng mellem afgrødens jordtildækning som følge af ukrudtsharvning og afgrødens højde på harvetidspunktet.

I det samme markforsøg som nævnt overfor blev fire scenarier undersøgt for anvendelse af sensor-baserede målinger af reflektans, vegetationens dækningsgrad og canopystrukturen i tidlige vækststadier med henblik på at estimere det potentielle udbytte af afgrøden. Resultaterne viste at ved at anvende 14 reflektansmålinger gennem hele vækstsæsonen, ni målinger af vegetationsdækket, to målinger af afgrødens canopystruktur samt ukrudtets tæthed i en multivariat ordinationsteknik under anvendelse af patial least squares (PLS) var denne metode i stand til at forklare 65% af udbyttevariationen med syv principal komponenter.

Ved at udelukke ukrudtets tæthed og canopystrukturen blev PLS-modellens evne til at prediktere udbyttet ikke forringet. Ved at anvende sensorbaserede målinger før vækststadie 21-22 var PLS-modellen i stand til at forklare 38% af udbyttevariationen. Yderligere reduktioner i antallet af målinger reducerede modellens nøjagtighed. Vi fandt dog at en enkelt måling gennemført 16-18 dage efter såning alene forklarede 27% af udbyttevariationen. Sammenlignet med anvendelse af alle tilgængelige måleresultater kan en tidlig sensorbaseret måling give rimelige estimater af det forventede udbytte, som kan hjælpe landmanden til at optimere anvendelsen af herbicider.

**Nøgleord:** Konkurrence, afgrøde-ukrudt interaktioner, *Hordeum vulgare* L., billedanalyse, mekanisk ukrudtsbekæmpelse, miksede lineære modeller, multivariat statistik, reflektans, vårbyg sorter, sortsforskelle.

## List of supporting papers

Paper I. Hansen, P.K., Kristensen, K. & Willas, J. (2008). A suppressive index for spring barley (*Hordeum vulgare* L.) varieties. Weed Research **48:**225-236.

Paper II. Hansen, P.K., Rasmussen, I.A., Holst, N. & Andreasen, C. (2007). Tolerance of four spring barley (*Hordeum vulgare* L.) varieties to weed harrowing. Weed Research **47:**241-251.

Paper III. Hansen, P.K. & Holst, N. (2008) Using multi temporal sensor based measurements in early growth stages to estimate potential yield of spring barley (*Hordeum vulgare* L.) varieties in competition with weeds. Manuscript.

Paper IV. Hansen, P.K. (2005) Tolerance to weed harrowing in spring barley genotypes. Poster 156 presented at the 13th European Weed Research Society Symposium, 19-23 June 2005, Bari, Italy.

Paper I and Paper II has been reproduced with kind permission of the publisher.

Below the papers will be referred to by their Roman numerals.

# Introduction

Increasing public awareness of unwanted side effects of the intense use of pesticides in agricultural production has increased the pressure to find alternative methods to control pests, fungi and weeds. Since 1987 three Pesticide Action Plans have focused on reducing pesticide use in conventional agriculture in Denmark (Anonymous, 2000b). As herbicides constitute the major part of the pesticide use (Anonymous, 2007), herbicide reduction will contribute towards a more sustainable production. As no herbicides are used in organic agriculture, an increase in the organically grown area or the use of some organic weed control methods in conventional agriculture will reduce the use of herbicides.

In contrast to conventional agriculture, in which weed control often is conducted curatively, effective control of weeds in cereals grown in organic or other low-input systems must rely on both preventative and curative methods in an integrated way (Bond & Grundy, 2001). Preventive methods include strategic use of diversified crop rotations (Melander *et al.,* 2005), placement of fertilisers (Rasmussen & Rasmussen, 1999) and use of varieties with strong competitiveness against weeds (Pavlychenko & Harrington, 1934; Christensen, 1995; Lemerle *et al.,* 1996). The curative control includes methods like pre- and post-emergence weed harrowing (Rasmussen, 1993).

A competitive crop can be defined as one that maintains its yield in presence of weeds (tolerant of competition; Goldberg (1990)) or as one that is able to reduce weed growth (suppress competitors; Tilman (1990)). In the latter definition one might distinguish between short and long term effect as the short-term effect (within one growing season) only influences the actual crop yield, but in the longer term the use of a strong competitor will reduce the weed seed production and thereby reduce problems in following crops. In this thesis the definitions by both Goldberg (1990) and Tilman (1990) were used, meaning that crop traits concerning increased competitiveness must 1) increase crop resource capture, 2) decrease weed resource capture and 3) have neutral or positive effects on the resources used by the crop (Caton *et al.,* 2001).

This thesis deals with different aspects of crop-weed interactions and how these interactions can be manipulated by utilise varieties with better competitiveness and tolerance to weed harrowing under organic or low-input growing conditions. Further it deals with the development of new methods with sensors to estimate the effect and outcome of the interactions between weeds and spring barley (*Hordeum vulgare* L.).

*Varietal differences in competitiveness against weeds*

In the last decades several studies have shown that there are strong differences between the competitive ability of cereal varieties (as reviewed by Lemerle *et al.,* 2001a) and that this effect is even more pronounced, when reduced doses of herbicides are applied (Christensen, 1994). However these studies have also shown that competitive ability cannot be attributed to a single growth character but to the total effect of several characteristics. In general, competitiveness of a crop is associated with quick emergence (Didon, 2002), rapid and abundant tillering (Lemerle *et al.,* 1996), high leaf area index (LAI) (Huel & Hucl, 1996; Seavers & Wright, 1999) and large canopy height (Wicks *et al.,* 1986; Christensen, 1995). In a study of six spring barley varieties grown under organic conditions, Didon and Hansson (2002) found that the most competitive varieties transmitted the least photosynthetically active radiation through the canopy during tillering and elongation and had high numbers of internodes. Didon (2002) showed that competitive varieties had an early stem elongation and the length of the two first internodes as well as the length of the main shoot was important morphological traits for competitiveness. Leaf angle also influences varietal competitiveness (Eisele & Köpke, 1997b). Davies *et al.* (2004) found that varieties with planophile leaves compensated for lack of canopy height, so that relatively short varieties with planophile leaves could be as competitive as tall varieties with erectophile leaves. By simulation of interactions between winter wheat (*Triticum aestivum* L.) and ryegrass (*Lolium multiflorum* L.), Olesen *et al.* (2004) showed that differences in the extinction coefficient were important parameters for the weed suppressive ability of eight different varieties and one variety mixture. The extinction coefficient describes the relative reduction of light flux through the canopy due to light absorption and is linearly correlated with leaf angle under certain assumptions (Goudriaan, 1988; Olesen *et al.,* 2004). The crop traits that affected weed suppression most in the simulations by Olesen *et al.* (2004) were early crop development, rapid growth in height and rapid growth in specific leaf area.

Cousens and Mokhtari (1998) concluded that a robust measure of competitiveness is needed if competitiveness is to be introduced as a varietal character besides other characteristics; yield potential, risk of lodging, resistance against diseases and so on. Lemerle *et al.* (2001a) agreed: “Ultimately, growers would like to be provided with a ranking of wheat varieties for competitive ability, as part of the normal varietal characteristics. This would enable the grower to choose strongly competitive varieties, a relatively low-cost management option, where weeds are expected to be a problem.” However, in a regular screening programme for variety competitiveness only a few, preferentially non-destructive, measurements of the most important growth traits can be afforded. Therefore, there is a need for a method to estimate the ranking of varietal competitiveness against weeds in a robust and cost-effective manner.

*Tolerance to weed harrowing*

Under organic or low input growing conditions weed control is often done by weed harrowing where spring tines of the harrow control weeds by uprooting and/or covering small weeds plants with soil (Kurstjens & Kropff, 2001). Pre- and post-emergence weed harrowing is often used in combination in organically grown spring cereals. Timing is important for the success of pre-emergence weed harrowing, because it should be conducted just before crop emergence to ensure an effective weed control without harming the crop plants (Rasmussen & Rasmussen, 1999). The efficacy of post-emergence weed harrowing relies on its selectivity, which has been defined as the relationship between the positive effect of weed control and the negative effect due to crop cover (Rasmussen, 1992). If the weed plants are large relative to the crop plants selectivity is reduced and the risk of damaging the crop mechanically or by soil coverage is increased (Rasmussen, 1991). The risk of crop damage also increases with the intensity of weed control, which is determined by the speed or aggressiveness of the spring tines (Kurstjens & Kropff, 2001). Crop damage due to weed harrowing has been shown to reduce yield (Kirkland, 1994; Rasmussen & Svenningsen, 1995; Jensen *et al.,* 2004). Apart from the direct effect on yield through changes in crop growth, indirect effects through altered conditions for crop-weed competition may be important, as the growing conditions of the weeds are reduced.

*Tolerance* to weed harrowing has been defined as the combined characteristics of the crop to *resist* initial damage caused by weed harrowing and to *recover* from this damage (Gundersen *et al.,* 2006). Resistance to initial damage is related to the height of the crop as well as the flexibility and shape of the leaves (Kurstjens & Perdok, 2000). A crop with high *recovery* is characterised by growth traits well-suited to overcome soil covering and maintain yield. The degree of realised recovery from soil covering depends on burial depth, soil texture and plant recovery processes (Baerveldt & Ascard, 1999; Kurstjens & Kropff, 2001).

Lemerle *et al.* (2001b) describe several studies showing strong varietal differences in weed suppression. The majority of these studies were conducted as a comparison between weedy and weed-free (herbicide treated) conditions. Only a few studies were conducted to estimate varietal differences in response to weed harrowing in cereals and to study if weed harrowing interacts with weed suppressive ability.

*Sensor-based measurement of early growth to predict yield*

In spring barley, the major part of the weed control is conducted before growth stage 21-23 BBCH (Lancashire *et al.,* 1991). At that time it is difficult to predict the eventual crop yield. Knowledge of potential crop yield is necessary to assess the yield loss potentially caused by weeds. The main difficulty of predicting crop yield early on in the season reason is that the weather in the remaining, major part of the growing season is decisive for yield formation. Nevertheless, this is the ideal time for optimising weed control, while weeds are small and crop-weed competition has hardly begun.

Several decision support systems uses weed density and growth stage at the time of weed control as input for decision algorithms, e.g. Crop Protection Online (Anonymous, 2005a) or WeedSoft (Hock *et al.,* 2006). Weed density and crop yield loss have been shown to follow a robust hyperbolic relation (Cousens, 1985) with asymptote and slope dependent on weed species, its growth stage and the crop (Holst, 2005). But weed density is laborious to assess, especially when one is aiming at site-specific weed management. Therefore it would be useful if it was possible to achieve a reliable crop yield estimate before weed control by automatic non-destructive sensor-based measurements in the early season.

In weed-free crops it is possible to use non-destructive measurements in late growth stages for predicting the yield by reasonable accuracy (Hansen *et al.,* 2003). Under weedy conditions several researchers have attempted to predict yield loss due to weeds by measuring the relative weed/total leaf area in the early growth stages (Lotz *et al.,* 1992; Kropff *et al.,* 1995; Lotz *et al.,* 1996; Ngouajio *et al.,* 1999c). This method gives a better description of the yield loss due to weeds compared to the density model, especially when the weeds emerge in flushes. However, there are some complications in using the method: 1) there is a need for *automatic* data acquisition to distinguish leaf area of crop and weeds, which is not possible in cereals yet, 2) the leaf area of the weeds must be combined with information of which species are present and the relative distribution of the species, as the competitive ability of weed species differs, 3) the method has till now proved too inaccurate yield loss predictions in sugar beet (*Beta vulgaris* L.) and spring wheat (*Triticum aestivum* L.) in competition with *Sinapis alba* L to be used in decision-making systems for integrated weed management (Lotz *et al.,* 1996).

Some researchers have used image analysis to measure relative leaf cover (the vertical projection of plant canopy on the ground) in maize (Ngouajio *et al.,* 1999a; Ngouajio *et al.,* 1999b), sugar beets (Heisel *et al.,* 2002) and vegetables (Grundy *et al.,* 2005). Other methods to discriminate crop and weed include chlorophyll fluorescence profiles (Keränen *et al.,* 2003), indices of plant reflectance spectra (Wiegand *et al.,* 1990; Vrindts *et al.,* 2002) and advanced image analysis methods (Andreasen *et al.,* 1997; Søgaard, 2005) as reviewed by Gerhards and Christensen (2003). All these methods use optoelectronic sensors or CCD cameras to measure reflectance in the green, red and often also near-infrared (NIR) wave lengths. Green leaves are characterised by a high reflectance in green and near-infrared wavelengths and low reflectance in the red spectrum compared with the reflectance from bare soil.

By combining output from different types of sensors, i.e. sensor fusion, measurement quality can be improved for instance for fruit quality assessments (Steinmetz *et al.,* 1999) or for monitoring sprayer boom movements (Ooms *et al.,* 2002) The statistical method used for prediction analysis must be able to handle multivariate data structures with high covariance and redundancy. This requirement is fulfilled by partial least squares regression (PLS) models (Rännar *et al.,* 1995; Kenkel *et al.,* 2002).

*The Ph.D. project was a part of BAR-OF*

This experimental base for this thesis was a part of the project "Characteristics of Spring Barley Varieties for Organic Farming, BAR-OF", which was a corporation between the Danish Technical University (formerly Risø National Laboratory), the University of Copenhagen, Faculty of Life Sciences (formerly The Royal Veterinary and Agricultural University) and the University of Aarhus, Faculty of Agricultural Sciences (formerly the Danish Institute of Agricultural Sciences) and the Danish Ministry of Food, Agriculture and Fisheries, the Danish Plant Directorate, Department of Seed. The project was grant-aided by the Danish Research Centre for Organic Farming (DARCOF) (http://www.darcof.dk).

The aims of BAR-OF were to provide experimental data, statistical analyses and modelling to fulfil five main objectives:

- To identify combinations of plant characteristics required for a barley crop to be successful in organic growing systems and develop methodologies for measuring these characteristics.
- To evaluate, by investigating genotype-environment interactions, the need for specific variety trials for organic farming, and if necessary implement such trials.
- To improve yield and yield stability in different organic farming systems by strategic use of the appropriate varieties and variety mixtures.
- To investigate the potential of different variety mixtures for reducing diseases and weeds and increasing nutrient uptake efficiency.

- To obtain new knowledge on plant competition, disease complexes, epidemiological models, nutrient acquisition and associations between molecular markers and agronomic traits.

These objectives was reached by the combined effort of scientists with different expertise within official variety testing, weed biology, plant pathology, plant nutrition, plant genetics, plant breeding, population biology, biostatistics and mathematic modelling. BAR-OF was structured by some central Work Packages (WPs); *variety testing*, which was conducted under both organic and conventional conditions, *mathematical modelling* of genotype-environmental interactions and analyses of whether *molecular markers* could be used to identify important agronomic traits in the varieties. As a supplement to these central WP's, three specific projects were conducted; examining *nutrient acquisition*, *disease complexes* and *crop-weed interaction*. This thesis was a part of the latter work package.

## Objectives

The objective of this thesis was to provide new knowledge about competition between varieties of spring barley and weeds, and the use of sensors to measure the competition. The main questions addressed were to

1. Determine the ranking and the differences among 79 varieties with regard to suppression of weed cover when the weed suppressive ability was measured in two ways:
   a. Directly, by weed cover measurements under weedy conditions at three different simulated organically grown locations in Denmark from 2002 to 2004
   b. Indirectly, by using sensor measurements of the varietal growth traits (early growth rate, leaf angle and culm length) measured under weed-free conventional conditions at 17 other experiments in Denmark from 2001 to 2003.

   The aim was to derive a low-cost and robust method for estimating weed suppressive ability to be used in the official variety testing.
2. Investigate the tolerance of four spring barley varieties to weed harrowing under organic growing conditions at two nutrient levels. The weed harrowing strategy was a combination of one pre- and one post-emergence weed harrowing. The effect of weed harrowing was characterised
   a. by soil covering of the varieties just after harrowing,
   b. by yield.

The aim was to investigate the possible interactions among variety, weed harrowing and weed suppression.

3. Investigate four scenarios of using sensor-based reflectance, cover and canopy structure measurements in the early growth stages to estimate the potential yield of four pure spring barley varieties and four variety mixtures of spring barley grown in competition with weeds under two different nutrient levels.
   a. How accurate can yield be predicted when all available non-destructive measurements from sowing until harvest are used,
   b. How accurate can yield be predicted when sensor-based measurements before growth stage 21-22 (Lancashire *et al.,* 1991) are used,
   c. If two measurements are possible before GS 21-22, which times are the best?
   d. If only one measurement is possible before GS 21-22, which time is the best?

## Materials and methods

The data used in the thesis originated from two sources: either some large variety field trials shared by all participants of the BAROF project (WP1) at three locations in Denmark or a specific field trial meant for studies of interactions between varietal competitiveness, nutrient levels and weed control (WP2). The latter field trail was only conducted at Research Centre Flakkebjerg.

Prior to establishment, all field trials at Research Centre Flakkebjerg were planned and designed in a Geographic Information System (GIS) software application called MarkGIS (Anonymous, 2000a) to be used in ArcView 3.2 (Anonymous, 2002a). The positions of the experiments were transferred and staked out in the field (Fig. 1) by a GPS RTK-controlled Trimble 5800 (Anonymous, 2002b) before nutrient application and sowing.

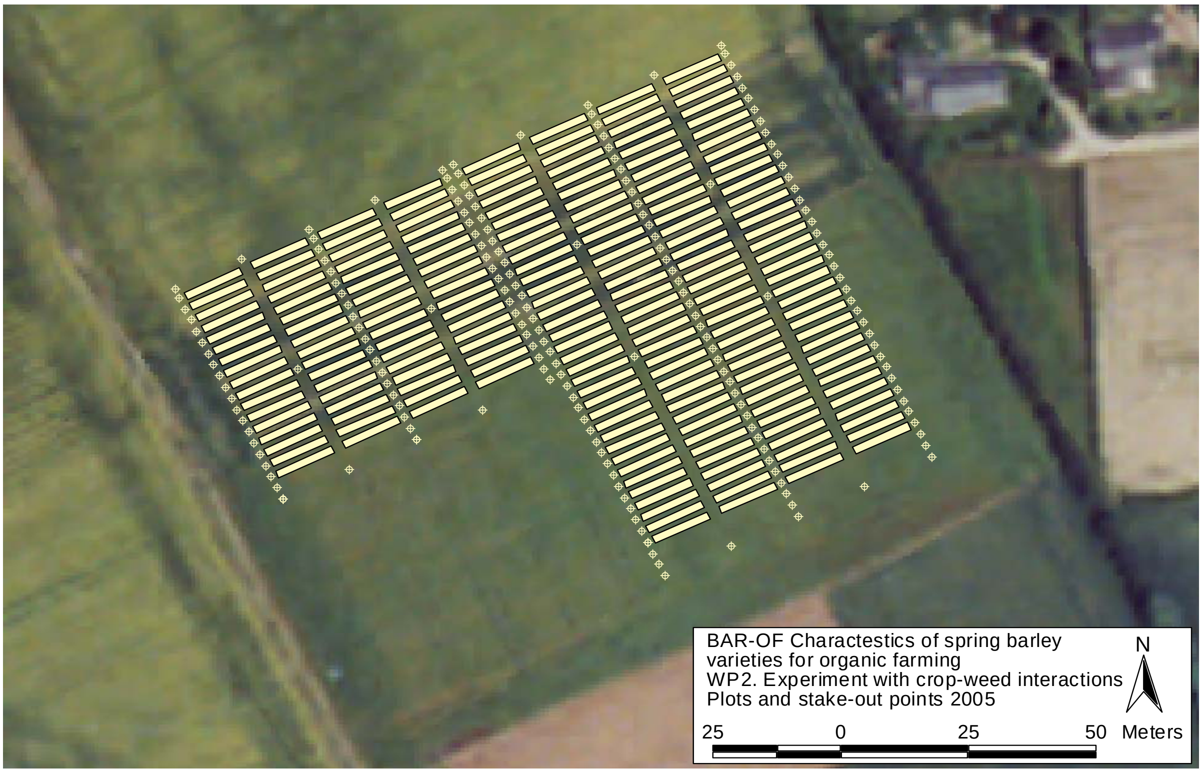


**Fig. 1** Example of use of MarkGIS in planning and staking out the field trial in WP2 in 2005. Yellow squares show plots in the experiment from 2005 and the points show where sticks are placed helping the tractor driver having the right direction when establishing the field trial. The aerial photo used as background is taken in 1999.

*WP1 field trials*

The data for the study described in Paper I originated from two types of experiments: 1) assessments of cover of surviving weeds after weed harrowing under 'organic' growing conditions and 2) growth parameters measured under conventional weed-free growing conditions. Three years (2002, 2003 and 2004) of experiments were conducted under simulated organic conditions; the experimental fields were grown according to the Danish rules for organic agriculture three to five years before the experiments but without formal certification. The experiments were repeated at three locations; Flakkebjerg (sandy loam), Foulum (loamy sand) and St. Jyndevad (coarse sand). The experiments included 123 different varieties and variety mixtures in 2002, 132 in 2003 and 48 in 2004.

For the purpose of validation of the developed model, weed coverage was recorded in two field trials in 2005; one at Foulum (35 varieties and mixtures) and one at an organic, certified farm in Dalmose (sandy loam) with 43 varieties and mixtures.

The experiments were planned as $\alpha$ designs (Patterson & Williams, 1976). The $\alpha$ designs can be regarded as generalisations of the more traditional lattice designs where each replicate is subdivided into a number of blocks in order to minimise the within block variation and thus improve the comparison of varieties. In the α-designs each replicate is also subdivided into a number of incomplete blocks, but the size of the block may be chosen within a wide range of block sizes. The α designs are thus more flexible because of the freedom to choose an appropriate block size and because they are available for almost any number of varieties. In the experiments in WP1 there were 6-8 varieties in each incomplete block. Plot sizes ranged from 11.3 to 16.5 $m^2$ across locations and years. The varieties were sown at 350 viable seeds $m^{-2}$ using a cone seeder. Three replicates were used in 2002 and 2003 but only two in 2004 as another treatment was included in the experiments.

Mechanical weed control included one pre-emergence weed harrowing and 1-3 post-emergence weed harrowings. At Jyndevad the crop was sown earlier than at Flakkebjerg and Foulum in all three years, and it was the only location where irrigation was used. Nutrients were applied as manure slurry with amounts equal to 40% in 2002 and 60% in 2003 and 2004 of the recommended levels according to crop rotation, soil type and location (Anonymous, 2002c; Anonymous, 2003). The level of applied nitrogen ranged between 79 and 92 kg total N $ha^{-1}$ (66 to 79 kg ammonium N $ha^{-1}$). Further information regarding measurement and statistical treatment of the data can be found in Paper I.

*WP2 field trials*

Data used in Paper I, Paper III and Paper IV, originated from a field trial with four varieties of spring barley (1=Modena; 2=Otira; 3=Orthega; and 4=Brazil) and three two-component mixtures (5=50% Modena + 50% Otira; 6=50% Modena + 50% Orthega; 7=50% Modena + 50% Brazil) and one three-component mixture (8=33% Modena + 33% Otira + 33% Orthega) of the varieties, representing the range in weed suppressiveness among varieties in the Danish variety list (Anonymous, 2005b). The varieties were studied in field trials only at Research Centre Flakkebjerg in 2004 and 2005. The crop rotation of the experimental areas is shown in Table 1. The soil was mouldboard-ploughed to a depth of 25 cm in late autumn.

**Table 1** Crop rotation in experimental fields prior to experiment

| Year | 2004 | 2005 |
|---|---|---|
| 2000 | Oats (*Avena sativa* L.) | |
| 2001 | Spring barley with undersown white clover (*Trifolium repens* L.) | Lucerne (*Medicago sativa* L.) |
| 2002 | White clover for seed production | Lucerne |
| 2003 | Winter rape (*Brassica napus ssp. napus* L.) | Oats |
| 2004 | Spring barley, experiment | Winter wheat (*Triticum aestivum* L.) |
| 2005 | | Spring barley, experiment |

The weather conditions in 2004 and 2005 were much alike (Table 2). Mean temperature in July 2004 was lower compared to July 2005, while August 2004 was warmer compared to August 2005. Growing degree-days (d°C), accumulated from the date of sowing with a base temperature of 0 ºC, were used as a timescale in Paper I and Paper IV. The interval from sowing to harvest was 1724 d°C in 2004 and 1660 d°C in 2005. Regarding precipitation, May 2004 was drier compared to the same month in 2005. By contrast, the rest of the growing season 2004 was more wet compared to 2005, especially in August, where the precipitation in 2004 was 50 mm higher than in 2005. Generally there was no or little effect of the nutrient level in 2004, which can be explained by relatively dry growing conditions in May compared to 2005.

**Table 2** Weather conditions at Flakkebjerg in the growing seasons of 2004 and 2005. Starting date of the accumulations were the sowing date, 15 April 2004 and 13 April 2005, ending date was 31 August each year

| | Accumulated daily mean temp, d°C | | Accumulated precipitation, mm | |
|---|---|---|---|---|
| Month | 2004 | 2005 | 2004 | 2005 |
| April | 159.1 | 142.6 | 5.6 | 7.6 |
| May | 349.3 | 343.1 | 30.0 | 43.5 |
| June | 399.8 | 405.7 | 69.3 | 48.0 |
| July | 456.9 | 526.8 | 89.2 | 69.1 |
| August | 540.2 | 491.9 | 89.5 | 39.0 |

The varieties were studied in a split-plot design. Whole plots consisted of the eight combinations of three factors: two levels each of herbicides (±), weed harrowing (±) and nutrient level

(40% or 80% of the recommended nitrogen need (Anonymous, 2003). Nutrients were applied as pig slurry injected into the soil. The eight subplots were arranged in two neighbouring rows with four subplots per row. Each subplot consisted of the four varieties and the mixtures. As the experiments in WP1, an α design was used to optimise the comparisons between varieties within whole plots (Patterson & Williams, 1976). With three replicates, there were 192 plots each year. The gross plot size was 2.5×14.5 $m^2$ and the net plot size was 1.50×12.0 $m^2$. The net plots were split into a part used for non-destructive measurements and combine harvesting (1.5×9.5 $m^2$) and a part used for destructive measurements (1.5×2.5 $m^2$).

The crop was sown with a seed drill with 12.0 cm row spacing on 15 April 2004 and 13 April 2005. Seed rates were adjusted for seed weights and germination rate to give a population of 350 plants $m^{-2}$. As model weeds a mixture of 25% viable seeds of *Chenopodium album* L., 25% *Phaselia tanacetifolia* Benth*.,* 25% *Brassica napus ssp. napus* L. and 25% *Trifolium incarnatum* L. *cv.* Poppelsdorfer was used in plots with no pesticide treatments. The weeds were sown 16 April 2004 and 13 April 2005 at a density of 200 seeds $m^{-2}$. The naturally occurring weeds were *Stellaria media* (L.) Vill., *Sinapsis arvensis* L., *Viola arvensis* Murray*, Veronica arvensis* L.*, Thlaspi arvense* L. and *Fallopia convolvulus* (L.) Å. Löve. The total density of these species did not exceed 50 plants $m^{-2}$, and the biomass of these species was included in the total weed biomass. Due to heterogeneous infestations of *Cirsium arvense* (L.) Scop. in the experiments the density of this species was recorded at 6 July 2004 (992 d°C) and 1 August 2005 (1435 d°C).

In the herbicide-treated plots, we applied a mixture of 7.5 g tribenuron-methyl $ha^{-1}$ (Express ST, 500 g a.i $kg^{-1}$, DuPont), 108 g fluroxypyr $ha^{-1}$ (Starane 180, 180 g a.i. $l^{-1}$ Dow AgroSciences) and 150 g surfactant $ha^{-1}$ (Lissapol Bio, 1000 g a.i. $l^{-1}$, Syngenta Crop Protection) on 12 May 2004 (310 d°C). In 2005 (13 May 2005, 265 d°C) we applied a mixture of 24 g ioxynil + 24 g bromoxynil $ha^{-1}$ (Oxitril CM, 200g + 200g a.i. $l^{-1}$, Bayer CropScience), 0.0255 g mefenpyr-diethyl $ha^{-1}$ + 0.0085 g iodosulfuron-methyl-Na $ha^{-1}$ (Hussar, 150g a.i.$kg^{-1}$ + 50g a.i.$kg^{-1}$, Bayer CropScience) + 400 g surfactant $ha^{-1}$ (Isoblette, 1000 g a.i. $l^{-1}$, Bayer CropScience). The applications were performed at a dosage of 150 l $ha^{-1}$ with nozzle type S-ISO-LD-02-110 (Hardi International, Helgeshøj Allé 38, Taastrup, Denmark) and a pressure of 230 kPa. Driving speed was 6 km $h^{-1}$. All dosages and mixtures were determined using the decision support system Crop Protection Online (Anonymous, 2005b). We assumed no interactions between herbicide treatments and the growth and development of the varieties.

One pre-emergence weed harrowing was conducted on 25 April 2004 (129 d°C) and 21 April 2005 (79 d°C). The driving speed was approximately 9 km $h^{-1}$. On 13 May 2004 (319 d

°C, crop growth stage (GS 21-22; Lancashire *et al.,* (1991)) and 17 May 2005 (300 d°C, crop GS 21-25) one post-emergence weed harrowing was conducted with a driving speed of approximately 7-8 km $h^{-1}$. The weeds were between cotyledon stage to four true leaves. The intensity of harrowing was adjusted by driving speed in an attempt not to exceed 20% crop burial at the post-emergence weed harrowing as an average. Weed harrowing was carried out with a spring-tine harrow (Einböck, Dorf an der Pram, Austria). The post-emergence weed harrowing in 2004 was done on humid soil, while the soil was dry on the surface in 2005. In both years pre- and post-emergence weed harrowing were conducted under sunny and windy conditions resulting in fast drying of the soil.

In each of the plots two sites were marked at emergence to ensure that the sensor-based measurements as well as weed density recordings were conducted at the same place every time. Further information regarding measurement and statistical treatment of the data can be found in Paper I, Paper III and Paper IV.

# General results and discussion

*Methods used in the studies*

Due to the fact that this thesis is based on experiments that were a part of the multidisciplinary BAROF project, certain possibilities of studies were opened and others were closed. Throughout the planning of the project, the degree of holism or wholeness-orientation in the chosen research methods was discussed, as holism is an extra criteria of scientific quality when the research subject is organic agriculture (Alrøe & Kristensen, 2002). However, when conducting and analysing results from holistic research, extra interactions will be introduced into the experiments, which might make it harder to make global conclusions about details in the experiment.

An example: the original design of the field trials in WP2 excluded interactions between varieties and foliar diseases by a generally application of fungicides, as the aim was to study crop-weed competition. However, as the trials were placed in the organic workshop area at Research Centre Flakkebjerg, where the use of non-organic methods, like a fungicide treatment is only allowed as a treatment aiming at comparison with other treatments, a general fungicide application was not allowed, and thereby interactions between varieties and their disease resistance was introduced into the experiments. Monitoring of the disease level was therefore needed, which was done by Mogens Støvring Hovmøller, the University of Aarhus, Faculty of Agricultural Sciences, Department of Integrated Pest Management. An analysis of the interactions showed, however, that in 2005 there was a significant interaction between weed harrowing and the amount of the foliar diseases powdery mildew (*Erysiphe graminis,* Table 3) and leaf spot (*Drechslera teres,* Table 4), as weed harrowing increased the disease level. However this was not the case in 2004 despite the more humid climate this year (Table 2).

**Table 3** Pwdery mildew (*Erysiphe graminis*) in herbicide untreated plots. Third-root transformed Least Significant Means values derived from model (2) in Paper I. Standard errors of the estimates were 0.10 in 2004 and 0.05 in 2005. Bold figures show significant differences between treatments within each year

| | 25.6.2004 | | 20.6.2005 | |
|---|---|---|---|---|
| Variety/mixture | Not harrowed | Harrowed | Not harrowed | Harrowed |
| Modena | **0.01** | **0.22** | 0.11 | 0.14 |
| Otira* | 0.04 | 0.03 | -0.00 | 0.03 |
| Orthega | **0.51** | **0.67** | 0.22 | 0.25 |
| Brazil | 0.74 | 0.69 | **0.21** | **0.52** |
| Modena+Otira | 0.09 | 0.10 | 0.01 | 0.04 |
| Modena+Orthega | **0.21** | **0.38** | **0.00** | **0.15** |
| Modena+Brazil | 0.14 | 0.16 | 0.11 | 0.07 |
| Modena+Otira+Brazil | 0.17 | 0.11 | **0.11** | **0.04** |

*MLO resistance

**Table 4** Leaf spot (*Drechslera teres*) in herbide untreated plots. Third-root transformed, Least Significant Mean values derived from model (2) in Paper I. Standard errors of the estimates were 0.08 in 2004 and 0.07 in 2005. Bold figures show significant differences between treatments within each year

| | 25.6.2004 | | 20.6.2005 | |
|---|---|---|---|---|
| Variety/mixture | Not harrowed | Harrowed | Not harrowed | Harrowed |
| Modena | 1.13 | 1.07 | 0.66 | 0.69 |
| Otira | 0.67 | 0.62 | 0.24 | 0.31 |
| Orthega | 0.35 | 0.34 | 0.15 | 0.19 |
| Brazil | **0.44** | **0.58** | **0.20** | **0.29** |
| Modena+Otira | **0.97** | **0.77** | **0.31** | **0.60** |
| Modena+Orthega | 0.68 | 0.70 | 0.44 | 0.38 |
| Modena+Brazil | 0.76 | 0.80 | **0.30** | **0.44** |
| Modena+Otira+Brazil | **0.72** | **0.52** | **0.27** | **0.35** |

In the design and stake-out of the experiments, a new method using a Geographic Information System (GIS) application in the design of the experiment in combination with GPS-RTK equipment to stake-out the experiment on the field was introduced. The advantage of using this method, which is inspired by precision farming systems, is that information about the experimental fields, stored in the GIS system, can be taken into account when placing the experiment on the actual field. This method can be useful if the field trials are aiming at more homogenous soil conditions inside each replicate or aiming at maximal heterogeneity or other requirements in the experimental design. However, the method showed strong advantages but needs further development concerning user interface before it is an appropriate method to use in general design and staking out of field trials.

*Varietal differences in competitiveness against weeds*

In Paper I, we developed a method for evaluating existing and new varieties with regard to weed suppressiveness. In this study we measured the weed suppressive ability of 79 varieties in two ways: 1) directly, by weed cover assessments under weedy simulated organically grown conditions, and 2) indirectly, by sensor measurements of varietal growth traits (reflectance, leaf angle and culm length) under weed-free conventional growing conditions. Based on the growth trait measurements, we could index the spring barley varieties with regard to weed suppressiveness, which ranged between 12% and 55% reduction of the maximal weed cover. A variety with medium suppressive ability was able to suppress the weed cover by about 30%.

The method developed in Paper I will be used for ranking the weed suppressiveness of existing and new varieties on the Danish variety list, which is available on www.sortinfo.dk. This information is mainly aimed at organic growers, as mechanical weed control is less efficient compared to chemical weed control, which means that weeds surviving the mechanical weed control need to be suppressed as much as possible, thereby reducing the possible increase in the weed seed bank. However conventional farmers, using spring barley as a cover crop for grass seed crops, can use this information as well to select varieties with reduced weed or in this case grass suppressive ability to provide the best growing conditions for the following grass seed crop. Weed suppressiveness will probably never become the most important factor in the choice of cultivar, but when some suitable varieties have been selected from the variety list based on yield potential, quality parameters, resistance to fungi and other agronomic traits, the weed suppressive index should also be taken into account.

*Tolerance to weed harrowing*

The aim of Paper I was to investigate the tolerance to weed harrowing of four spring barley varieties and to examine the possible interactions between varietal weed suppressive ability and nutrient level. We defined tolerance as the combined effect of crop resistance (ability to resist soil covering) and crop recovery (the ability to recover in terms of yield). The weed harrowing strategy was a combination of one pre- and one post-emergence weed harrowing.

In terms of yield, the four varieties responded significantly different to weed harrowing, and the response depended on nutrient level. At the lower nutrient level, weed harrowing caused an increase in yield of 4.4 hkg $ha^{-1}$ for a strong competitor (*cv.* Otira), while there was no effect on yield at the higher nutrient level. For a weaker competitor (*cv.* Brazil), weed harrowing caused no change in yield at the lower nutrient level, whereas yield decreased by 6.0 hkg $ha^{-1}$ at the higher nutrient level. There were marked differences between the weed suppressive ability of the four varieties when not harrowed, with less pronounced but significant differences when harrowed. However, weed harrowing did not change the weed suppressive ability of a variety. We found a negative correlation between cover caused by harrowing ($\Delta VC$) and canopy height, H (Fig. 2). Higher plants are covered less. Unfortunately, if plants of the same species are buried by soil (Kurstjens & Perdok, 2000), the high plants have a larger burial depth than sort plants.

In Paper IV (which was published before the experiment in 2005 was finished) a clear correlation between the degree of soil-coverage and the re-growth ($REIP\ d°C^{-1}$) in the three-week period after weed harrowing in 2004 was shown. The degree of soil coverage was measured as the difference between reflectance measured as Red Edge Inflexion Point (REIP) before and after weed harrowing *(ΔREIP*). (see Paper I for description of the calculation of REIP). The results presented in Paper IV did not show any correlation in 2003, as cover as well as re-growth was much smaller this year due to the fact that weed harrowing was conducted in later growth stages compared to 2004 and 2005. The results from 2005, however, showed the same tendency as in 2004 (Fig. 3), which means that varieties with relative short canopy (Fig. 2) are likely to have a stronger re-growths after weed harrowing (Fig. 3).

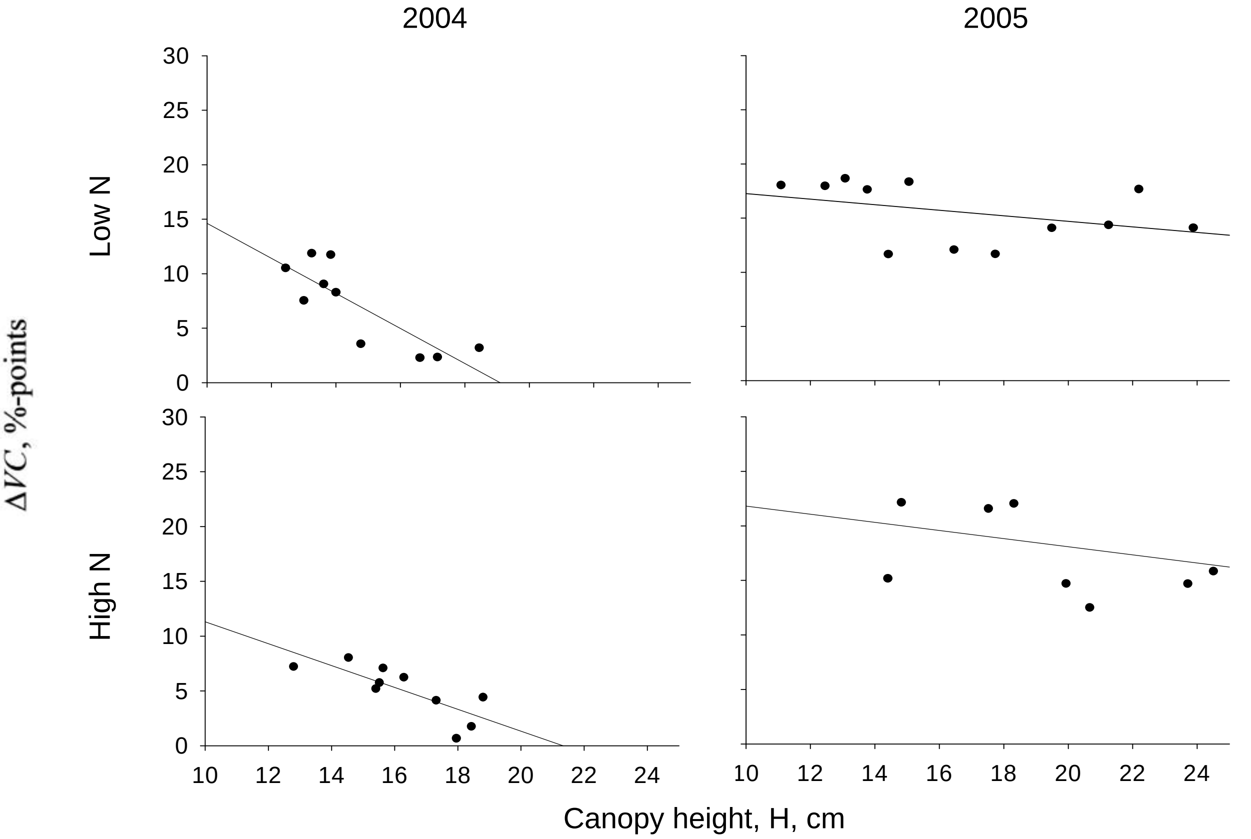


**Fig. 2** Relationship between canopy height, *H* and the vegetation cover due to harrowing, *ΔVC* under low (upper) and high (lower) nutrient levels in 2004 (left) and 2005 (right). Each point represents one plot. Low nutrients 2004:34.2-1.9*H* ($r^2$=0.69). Low nutrients 2005: 25.5-0.6*H* ($r^2$=0.14). High nutrients 2004: 24.0-1.2*H* ($r^2$=0.53). High nutrients 2005: 31.5-0.07*H* ($r^2$=0.26).

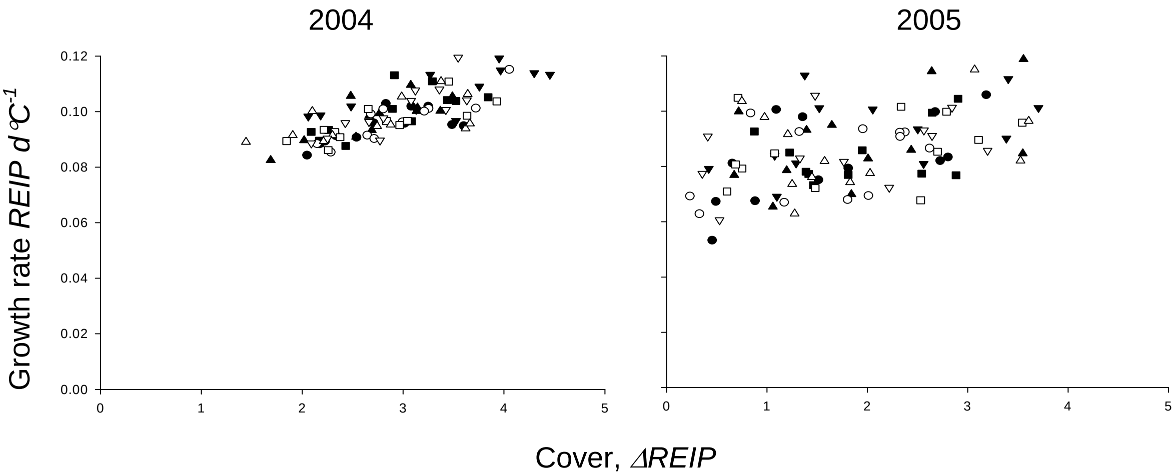


**Fig. 3** The relationship between the degree of soil coverage (ΔREIP) and the regrowth (REIP °Cd$^{-1}$) in the following three-week period after weed harrowing in plots treated chemically in 2004 (left) and 2005 (right). Modena (●), Otira (■), Orthega (▲) Brazil (▼), 50%Modena+50%Otira (○), 50% Modena + 50%Orthega (□), 50% Modena + 50% Brazil (Δ) and 33% Modena + 33% Otira + 33% Orthega (∇) in the three-weeks period after weed harrowing in plots treated chemically.

*Sensor-based measurement of early growth and multivariate statistics*

In Paper III, we used data from the same experiment as described in the previous paragraph to determine the possibilities of using non-destructive sensor-based measurement early in the growing season to determine the yield in plots without mechanical treatments. If possible, an early and precise prediction of the growth dynamics of both crop and weeds could give a more precise assessment of the need for weed control. This can lead to a better confidence to point out sites in the field where weed control can be reduced or even eliminated, because the competitive relationship between crop and weeds is in favour of the crop, and the potential yield gain achieved by weed control cannot pay the weed control costs.

Several studies of the competition between crop and weeds have shown that the quota of weed leaf area to the total leaf area (crop + weed) gives a more accurate prediction of the yield reduction caused by weeds, than the weed density (plants $m^{-2}$) (Kropff & Spitters 1991; Lotz *et al.,* 1996). This means that many small weed plants can have the same yield reducing effect as a few but large plants.

The results showed that by using 14 reflectance measurements conducted through the entire growing season as well as measurement of weed density and two canopy structure measurements, a multivariate ordination technique using partial least squares (PLS) was able to explain 65% of the yield variation with seven principal components (PCs). By excluding weed density and the canopy structure measurements the predictability of the PLS model was not reduced. By using only the first five sensor-based measurements (before crop growth stage 21-22), the PLS model could explain 38% of the yield variation. Further reductions in the numbers of measurements reduced the accuracy of the model; however we found that a measurement 16-18 days after sowing alone explained 27% of the variation in yield.

The results were based on the total leaf area measured in different ways as the image analysis algorithm did not distinguish between crop and weeds. However the results of the experiments have shown that it is possible to use a combination of image analysis and reflectance measurements to predict the interactions between crop and weeds. Compared to the explained variation by using all available measurements through the entire growing season, an early vegetation cover measurement can give reasonable estimates of the expected yield, helping the farmer to optimise the use of herbicides.

## General conclusion

The results of the present work have shown that

- it was possible to use sensor based measurements of varietal growth traits (reflectance, leaf angle and culm length) conducted under conventional weed-free growing conditions to predict the cover of weeds under organic conditions,
- the weed suppressive ability of 79 studied varieties of spring barley ranged between 12% and 55% reduction of the maximal observed weed cover in the experiments,
- the yield responses of four spring barley varieties were significantly different, when a weed harrowing strategy that combined one pre- and one post-emergence weed harrowing was applied. The response was dependent on the nutrient level,
- there were marked differences between the weed suppressive ability of the four varieties when not harrowed, with less pronounced but significant differences when harrowed, meaning that weed harrowing did not change the weed suppressive ability of the varieties,
- there was a significant correlation between soil covering caused by harrowing and canopy height of the varieties,
- by using 14 reflectance measurements conducted through the entire growing season, nine vegetation-cover measurements before GS 40 as well as measurement of weed density and two canopy structure measurements, the PLS model using a multivariate ordination technique was able to explain 65% of the yield variation with seven principal components,
- by excluding weed density and the canopy structure measurements the predictability of the PLS model was not reduced; by using only the first five sensor-based measurement (before crop growth stage 21-22), the PLS model was able to explain 38% of the yield variation and
- further reductions in the numbers of measurements reduced the accuracy of the model; however, it was found that a measurement 16-18 days after sowing alone explained 27% of the variation in yield, which means that an early sensor-based measurement of the vegetation can give reasonable estimates of the expected yield, helping the farmer to optimise the use of herbicides.

To get further insight in the subject of measuring and managing competition under organic or low input growing conditions, it is suggested that future work should focus on the following areas

- Further studies of the response of mechanical weed control on different genotypes, especially studying the interaction with nutrient level. The aim could be to identify genotypes suited for low or high input growing systems respectively, conducted with and without mechanical weed control.
- Further studies of the correlation between vegetation cover and light interception and LAI in the early growth stages as the results have indicated that the fusion of the two types of measurements may have a synergetic effect on the total predictive accuracy. It could be an advantage to include methods that are able to distinguish between crop species and genotype and the present weed species, i.e by using like the method described by Søgaard, (2005) to improve the predictability of the model. It will be necessary to combine the derived information with knowledge of growth rates of the individual species to improve the yield prediction.
- In the BAR-OF experiments, mixtures were included that were not considered in this thesis. There are, however, some indications that genotype mixtures may improve the weed suppressive ability (Kiær *et al.,* 2006) compared by the mean weed suppression of the pure varieties. This difference could be caused by niche differentiation. In future competition studies, this aspect could be of interest for a detailed study. For example, how different genotypes in a mixture compete, and how a strong competing mixture is composed: should the different genotypes in the mixture have the growth habit or should they be different?

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# Appendix
# Paper I. A weed suppressive index for spring barley (*Hordeum vulgare* L.) varieties

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## Summary

A screening programme for crop variety competitiveness would ideally be based on only a few, non-destructive measurements of key growth traits. In this study we measured the weed suppressive ability of 79 varieties of spring barley in two ways: 1) directly, by weed coverage assessments under weedy conditions at three Danish locations in 2002-2004, and 2) indirectly, by non-destructive measurements of varietal growth traits under weed-free conditions in 17 other experiments in Denmark in 2001-2003. Based on just four varietal growth traits (reflectance, leaf area index, leaf angle and culm length), we successfully developed a method for indexing the weed suppressive ability of spring barley varieties. The suppressive index ranged from 12% in 'Lux' and 55% in 'Modena' in proportion to the 90% quantile coverage of all varieties. The index was validated against independent data from two locations in 2005 with 14 and 24 varieties and found valuable for future use in regular screening programmes.

**Keywords:** cultivars, crop-weed interaction, competitive ability, variety testing, non-linear mixed modelling, weed suppression, sensor-based measurements, cereals

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## Introduction

Effective control of weeds in cereals grown in organic or other low-input systems must rely on both preventive and curative methods in an integrated way (Bond & Grundy, 2001). Preventive methods include placement of fertilisers (Rasmussen & Rasmussen, 1999) and diversified crop rotations (Melander *et al.,* 2005). Curative control includes methods like pre- and post emergence weed harrowing (Rasmussen, 1993). Additionally because cereal varieties differ in competitiveness against weeds (Pavlychenko & Harrington, 1934; Christensen, 1995; Lemerle *et al.,* 1996), choice of variety thus enters the suite of preventive methods.

A competitive crop can be defined (Goldberg, 1990) as one that maintains its yield in presence of weeds (tolerant of competition) or as one that is able to reduce weed growth (suppress competitors) (Tilman, 1990). In this study the latter definition is used. Weed suppressive ability cannot be attributed to a single growth trait but to the total effect of several different traits. In general, competitiveness of a crop is associated with quick emergence (Didon, 2002), rapid and abundant tillering (Lemerle *et al.,* 1996), high leaf area index (LAI) (Huel & Hucl, 1996; Seavers & Wright, 1999) and canopy height (Wicks *et al.,* 1986; Christensen, 1995). In a study of six spring barley varieties grown under organic conditions, Didon and Hansson (2002) found that the most competitive varieties transmitted the least photosynthetically active radiation through the canopy during tillering and elongation and had high numbers of internodes. Didon (2002) showed that competitive varieties had an early stem elongation and that the length of the two first internodes as well as the length of the main shoot were important morphological traits for competitiveness. Leaf angle also influences varietal competitiveness (Eisele & Köpke, 1997b). Davies *et al.* (2004) found that varieties with planophile leaves compensated for lack of canopy height, so that relatively short varieties with planophile leaves could be as competitive as tall varieties with erectophile leaves. By simulation of interactions between winter wheat (*Triticum aestivum* L.) and ryegrass (*Lolium multiflorum* L.), Olesen *et al.* (2004) showed that differences in the extinction coefficient were important for the weed suppressive ability of eight varieties and one variety mixture. The extinction coefficient describes the relative reduction of light flux through the canopy due to light absorption and is linearly correlated with leaf angle under certain assumptions (Goudriaan, 1988; Olesen *et al.,* 2004). The crop traits that affected weed suppression most in the simulations by Olesen *et al.* (2004) were early crop development, rapid growth in height and rapid growth in specific leaf area.

Cousens and Mokhtari (1998) concluded that a robust measure of competitiveness is needed if competitiveness is to be introduced as a varietal character besides other characteristics; yield potential, risk of lodging, resistance against diseases etc. Lemerle *et al.* (2001a) agreed: "Ultimately, growers would like to be provided with a ranking of wheat varieties for competitive ability, as part of the normal varietal characteristics. This would enable the grower to choose strongly competitive varieties, a relatively low-cost management option, where weeds are expected to be a problem." But how can we measure the competitiveness of the varieties in a cost-effective and robust way? In regular screening programmes for variety competitiveness only a few, preferentially non-destructive, measurements of the most important growth traits can be afforded.

Seavers and Wright (1999) suggested using a simple weighted average of important characters for weed suppression as a weed suppression index. In Denmark, rankings of a suppressive index for both spring barley (*Hordeum vulgare* L.) and winter wheat are updated annually as part of the Danish variety testing programme (Hansen, 2005). For spring barley this index has been calculated from two growth traits (LAI and culm length) measured under weed-free conditions late in the growing season. Hansen (2002) found that the index was able to predict the relative weed suppressive ability of eight pure varieties and one variety mixture with sufficient precision to be of practical use. However the index did not account for differences in early growth, and therefore it remained an open question whether including additional growth traits in the calculations would result in an improved index.

In this study, we determined the ranking of 79 varieties with regard to suppression of weed coverage, measured in two ways: 1) directly, by assessments of the coverage of surviving weeds under simulated organic growing conditions after mechanical weed control at three different locations in Denmark in 2002-2004, and 2) indirectly, by using measurements of the varietal growth traits (early reflectance, leaf angle, leaf area index and culm length) measured under weed-free conditions at 17 other experiments in Denmark in 2001-2003. A separate field study in 2005 provided validation data. Our aim is to derive a robust and low-cost method for estimating weed suppressive ability, for use in the official variety testing and in breeding programmes.

## Materials and methods

Two types of data were collected: 1) weed coverage, assessed under simulated organic growing conditions after mechanical weed control and 2) crop growth parameters measured under conventional weed-free growing conditions.

### *Weed coverage*

#### Experimental conditions

Three years (2002, 2003 and 2004) of experiments were conducted under simulated organic conditions; the experimental fields had been grown according to the Danish rules for organic agriculture three to five years before the experiments but without formal certification. The experiments were repeated at three locations; Flakkebjerg (sandy loam), Foulum (loamy sand) and Store Jyndevad (coarse sand). The experiments included 123 different varieties and variety mixtures in 2002, 132 in 2003 and 48 in 2004.

For the purpose of validation, weed coverage was recorded in two field trials in 2005; one at Foulum (35 varieties and mixtures) and one at an organic certified farm in Dalmose (55°17'N, 11°22'E; sandy loam) with 43 varieties and mixtures.

The experiments were planned as $\alpha$-designs (Patterson & Williams, 1976). The $\alpha$-designs can be regarded as generalisations of the more traditional lattice designs where each replicate is subdivided into a number of blocks in order to minimize the within block variation and thus improve the comparison of varieties. In the $\alpha$-designs each replicate is also subdivided into a number of incomplete blocks, but the size of the block may be chosen within a wide range of block sizes. The $\alpha$-designs are thus more flexible because of the freedom to choose an appropriate block size and because they are available for almost any number of varieties. In our experiments there were 6-8 varieties in each incomplete block. Plot sizes ranged from 11.3 to 16.5 $m^2$ across locations and years. The varieties were sown at 350 viable seeds $m^{-2}$ using a cone seeder. Three replicates were used in 2002 and 2003 but only two in 2004.

Mechanical weed control included one pre-emergence weed harrowing and 1-3 post-emergence weed harrowings (Table 1). At Jyndevad the crop was sown earlier than at Flakkebjerg and Foulum in all three years (Table 1), and it was the only location where irrigation was used. Nutrients were applied as manure slurry with amounts equal to 40% in 2002 and 60% in 2003 and 2004 of the recommended levels according to crop rotation, soil type and location (Anonymous, 2002c; Anonymous, 2003). The level of applied nitrogen ranged between 79 and 92 kg total N $ha^{-1}$ (66 to 79 kg ammonium N $ha^{-1}$).

**Table 1** Dates of sowing, weed harrowing, weed coverage assessment at the four locations in 2002-2005. The trials from 2005 were used as validation data

| Location | Operation | 2002 | 2003 | 2004 | 2005 |
|---|---|---|---|---|---|
| Jyndevad | | | | | |
| | Sowing | 12 April | 25 March | 31 March | - |
| | Pre emergence harrowing | 18 April | 08 April | 10 April | - |
| | Post emergence harrowing | 30 April | 23 April | 28 April | - |
| | " | 10 May | - | 11 May | - |
| | Weed coverage assessment | 09 June | 22 July | 28 June | - |
| Flakkebjerg | | | | | |
| | Sowing | 08 April | 25 April | 14 April | - |
| | Pre-emergence harrowing | 22 April | 05 May | 22 April | - |
| | Post-emergence harrowing | 13 May | 19 May | - | - |
| | " | 03 June | 27 May | - | - |
| | Weed coverage assessment | 25 June | 21 July | 23 June | - |
| Foulum | | | | | |
| | Sowing | 09 April | 07 April | 17 April | 18 April |
| | Pre-emergence harrowing | 19 April | 21 April | - | - |
| | Post-emergence harrowing | 08 May | 05 May | 29 April | 24 April |
| | " | 13 May | 09 May | 03 May | 12 May |
| | " | 22 May | - | 11 May | 18 May |
| | Weed coverage assessment | 03 July | 23 July | 30 June | 12 July |
| Dalmose | | | | | |
| | Sowing | - | - | - | 18 April |
| | Pre-emergence harrowing | - | - | - | - |
| | Post-emergence harrowing | - | - | - | 17 May |
| | " | - | - | - | 25 May |
| | Weed coverage assessment | - | - | - | 22 July |

## Measurements

Weed coverage (C, %) was assessed by the same person in June to July in all plots throughout the four years of experiments (Table 1). At each location, weeds were grouped into the four dominant dicotyledonous weeds (1-4), perennial weeds (5), and a remaining weed group (6) (Table 2). Only the summed coverages of group 1 to 4 and 6 were used in the analyses, as perennial weeds (5) were rare.

**Table 2** Average coverage of the 4 most frequent weed species and the group of other annual weed species. and the sum and 90% quantile of weed coverage $\widehat{C}_{\max,ly}$ at the four locations in the four years

| | 2002 | | 2003 | | 2004 | | 2005 | |
|---|---|---|---|---|---|---|---|---|
| Location | Species | C, % | Species | C, % | Species | C, % | Species | C, % |
| Jyndevad | *Polygonum spp.* | 8.5 | *Geranium spp.* | 5.4 | *Viola arvensis* | 7.7 | | |
| | *Stellaria media* | 5.3 | *Viola arvensis* | 4.4 | *Polygonum spp.* | 7.6 | | |
| | *Spergula arvensis* | 1.1 | *Chenopodium album* | 4.4 | *Chenopodium album* | 5.7 | | |
| | *Viola arvensis* | 0.3 | *Polygonum spp.* | 2.0 | *Geranium spp.* | 1.0 | | |
| | Other weed species | 4.5 | Other weed species | 2.9 | Other weed species | 4.2 | | |
| Sum | | 19.8 | | 19.1 | | 26.1 | | |
| $\widehat{C}_{\max,ly}$ | | 27.5 | | 27.4 | | 34.1 | | |
| Flakkebjerg | *Sinapis arvensis* | 5.8 | *Polygonum spp.* | 9.0 | *Polygonum spp.* | 18.0 | | |
| | *Chenopodium album* | 1.3 | *Matricaria spp.* | 0.9 | *Chenopodium album* | 12.6 | | |
| | *Polygonum spp.* | 3.1 | *Lamium spp.* | 0.9 | *Sinapis arvensis* | 4.8 | | |
| | *Matricaria spp.* | 0.1 | *Fumaria officinalis* | 0.3 | *Matricaria spp.* | 3.5 | | |
| | Other weed species | 1.5 | Other weed species | 5.1 | Other weed species | 3.7 | | |
| Sum | | 11.7 | | 16.2 | | 42.6* | | |
| $\widehat{C}_{\max,ly}$ | | 16.7 | | 27.2 | | 60.6 | | |
| Foulum | *Stellaria media* | 3.2 | *Polygonum spp.* | 10.9 | *Stellaria media* | 9.7 | *Galeopsis spp.* | 21.2 |
| | *Galeopsis spp.* | 2.6 | *Galeopsis spp.* | 7.4 | *Viola arvensis* | 4.0 | *Polygonum spp.* | 6.6 |
| | *Polygonum spp.* | 1.9 | *Stellaria media* | 7.3 | *Veronica spp.* | 3.8 | *Stellaria media* | 2.9 |
| | *Veronica spp.* | 0.1 | *Veronica spp.* | 1.7 | *Polygonum spp.* | 2.7 | *Chenopodium album* | 0.3 |
| | Other weed species | 1.2 | Other weed species | 1.6 | Other weed species | 5.8 | Other weed species | 1.0 |
| Sum | | 8.9 | | 28.8 | | 26.0 | | 31.9 |
| $\widehat{C}_{\max,ly}$ | | 12.5 | | 36.5 | | 41.0 | | 38.0 |
| Dalmose** | | | | | | | *Chenopodium album* | 2.6 |
| | | | | | | | *Polygonum spp.* | 1.0 |
| | | | | | | | Other weed species | 0.4 |
| Sum | | | | | | | | 4.0 |
| $\widehat{C}_{\max,ly}$ | | | | | | | | 10.8 |

* In this environment there were also a coverage of *Elymus repens* (3.7%) *Tussilago farfara* (1.7%) and *Cirsium arvense* (8.1%)
** Low species density limited the number of species.

### Statistics

To adjust for experimental design, the recorded sums of weed coverages in each combination of year and location (hereafter called environment) were analysed according to the experimental plan. We assumed that the effects of replicate and incomplete blocks within replicates were random:

$$C_{vmr} = \mu + \alpha_v + D_r + F_{mr} + E_{vmr} \tag{1}$$

where $C_{vmr}$ is the weed coverage of variety or mixture $v$, in incomplete block $m$ of replicate $r$, $\mu$ is the mean value and $\alpha$ is the effect of variety or mixture $v$. $D_r$ is the random effect of replicate $r$, $F_{mr}$ is the random effect of block $mr$ and $E_{vmr}$ is the residual variation. All random effects were considered independent and normally distributed with zero mean and constant variance.

Based on the model parameters we estimated weed coverage for each variety in each environment as

$$\widehat{C}_{vly} = \hat{\mu}_{ly} + \hat{\alpha}_{vly} \tag{2}$$

where $\widehat{\mu}_{ly}$ and $\widehat{\alpha}_{vly}$ are the estimates of $\mu$ and $\alpha_v$ from eqn. (1) for the trial at location $l$ in year $y$.

In the following analyses, we only included pure varieties that had been in the experiments for at least two years. This selection procedure resulted in 79 varieties in 2002 and 2003 and 24 in 2004. The number of these varieties available for validation was 14 for Foulum and 24 for Dalmose 2005.

Under the assumption of no interaction between weed harrowing and weed suppressive ability among the varieties (Hansen *et al.,* 2007b), the relative reduction in weed coverage, $\lambda_v$, for variety $v$ could be expressed as

$$\frac{\widehat{C}_{\max,ly} - \widehat{C}_{vly}}{\widehat{C}_{\max,ly}} = \lambda_v + E^*_{vly} \tag{3}$$

where $\widehat{C}_{\max,ly}$ denotes the 90% quantile based on all single plot observations of the weed coverage at location $l$ in year $y$. This term is used here as a measure of the maximal weed pressure. $E^{*}_{vly}$ describes the random variation. Isolation of variable $\widehat{C}_{vly}$ yields

$$\widehat{C}_{vly} = \left(1-\lambda_v\right)\widehat{C}_{\max,ly} + E^{*}_{vly} \tag{4}$$

To stabilise the variance of the recorded weed coverages, we square-root transformed both sides of eqn. (4) and estimated the variety suppression index $\lambda_v$ by maximum likelihood using the model:

$$\sqrt{\widehat{C}_{vly}} = \sqrt{\left(1-\lambda_v\right)\widehat{C}_{\max,ly}} + E^{**}_{vly} \tag{5}$$

where $E^{**}_{vly}$ describes the random variation. Other terms are as described above.

### *Crop growth parameters*

#### Experimental conditions

In 17 single-replicate experiments in the growing seasons 2001-2003 a number of spring barley varieties were tested at each location (Table 3). The soil types ranged from coarse sand at Jyndevad to clay at Rønhave. Varieties were sown using a cone seeder. The plot size was 1.6×10 m, and the plots were treated chemically to control weeds, pests and diseases. Nutrient levels followed the Danish recommendations according to crop rotation, soil type and expected nutrient leaching at each location (Deneken & Pedersen, 2001; 2002; 2003).

**Table 3** Locations and number of varieties each year for measurements of growth traits

| Year | Name and position of locations | No of varieties or mixtures in experiment |
|---|---|---|
| 2001 | Karise (55°19'N, 2°13'E) | 114 |
| | Refsvindinge (55°16'N, 10°41'E) | - |
| | Adamshøj (55°26'N, 11°51'E) | - |
| | Borris (55°57'N, 8°37'E) | - |
| | Tystofte (55°14'N, 11°1'E) | - |
| 2002 | Flakkebjerg(55°19'N, 11°24'E) | 119 |
| | Foulum (56°30'N, 9°36'E) | - |
| | Borris (55°57'N, 8°37'E) | - |
| | Jyndevad(54°54'N, 9°10'E) | - |
| | Holstebro (56°20'N, 8°27'E) | - |
| | Grindsted (55°46'N, 8°50'E) | - |
| | Rønhave (54°57'N, 9°46'E) | - |
| 2003 | Jyderup (55°34'N, 11°4'E) | 140 |
| | Karise (55°19'N, 2°13'E) | - |
| | Holstebro (56°20'N, 8°27'E) | - |
| | Tystofte (55°14'N, 11°1'E) | - |
| | Roskilde (55°37'N, 12°1'E) | - |

## Measurements

At growth stage (GS) 31 BBCH (Lancashire *et al.,* 1991), spectral light reflectance was measured with a specially designed hand-held instrument, with two random samples in each plot. The instrument consisted of a laptop computer (HP200LX, Hewlett-Packard, Singapore) and two sets of two-band-sensors (SKYE SKR 1800, Skye Instruments, Inc. 21, Ddole Enterprise Park, Llandrindod Wells, Powys LD1 6DF UK) connected to an A/D converter of type SDL2500 (Skye Instruments, Inc. 21, Ddole Enterprise Park, Llandrindod Wells, Powys LD1 6DF UK), all mounted on a stand (Kristensen, 1997). The sensors consisted of a pair of silicon photodiodes and specific interference filters that transmitted only red (650±10 nm) and near infrared (810±10 nm) light. One pair of sensors, which was hemispherical cosine corrected, was used to measure the incoming red (*R*) and near-infrared radiation (*NIR*). The other pair of sensors which had a limited field-of-view (FOV) of 26° was used to measure the reflected *R* and *NIR* from the canopy. The 26° FOV device was kept at a sensor height of 1.80 m and gave a circular measurement area of 0.50 $m^2$. The spectral measurements were converted to a mean value of the ratio vegetation index (*RVI*) for each plot, location and year by

$$RVI = \frac{R_{\uparrow,810} / R_{\downarrow,810}}{R_{\uparrow,650} / R_{\downarrow,650}} \quad (6)$$

where $R_{\uparrow,810}$ is the reflected NIR radiation, $R_{\downarrow,810}$ is the incoming NIR radiation, $R_{\uparrow,650}$ is the reflected red radiation, $R_{\downarrow,650}$ and is the incoming red radiation.

We measured leaf area index (LAI, $m^2$ $m^{-2}$), the fraction of diffuse non-intercepted radiation that reaches the soil surface (*DIFN*) and the mean tilt angle (*MTA,°*) at GS 65 with a LICOR-2000 Plant Canopy Analyser (LI-COR Biosciences, 4421 Superior St., Lincoln, NE 68504 USA) (Welles & Norman, 1991) as the average of two measurement sequences in each plot. One measurement sequence consisted of one measurement above the crop canopy immediately followed by one at the soil surface. Measurements from LAI-2000 were carried out under overcast conditions or in the evenings, as measurements are only reliable under diffuse lighting conditions. A *MTA* of 0° implies horizontal leaves, and 90° vertical leaves.

Culm length (*CL, cm)* was measured between GS 69 and GS 83 as the vertical distance between the soil surface and the base of the ear with one measurement in each plot.

## Statistics

Each of these measured growth traits (*RVI*, *CL*, *LAI*, *DIFN* and *MTA*) were analysed for all varieties and mixtures (Table 3) using the model,

$$X_{vLY} = \mu' + \omega_v + G_Y + H_{LY} + E^{\dagger}_{vLY} \quad (7)$$

where $X_{vLY}$ is the value of the growth traits (*CL*, *RVI*, *LAI*, *DIFN* or *MTA*) for all varieties and mixtures *v,* at location *L* in year *Y* (Table 3). $\omega_v$ is the effect of variety. $G_Y$ and $H_{LY}$ are the effects of year and the interaction between location and year, respectively. $E^{\dagger}_{vLY}$ is a term describing the residual variation. $G_Y$, $H_{LY}$ and $E^{\dagger}_{vLY}$ were all considered random, independent and normally distributed with a constant variance. All parameters of the model were estimated by restricted maximum likelihood.

Based on model parameters from eqn. (7), the standardised values (with a mean of 0 and a standard deviation of 1) of the growth trait were estimated for each pure variety that had been in the experiments for at least two years (79 varieties) as

$$\widehat{X}_v = \frac{\widehat{\omega}_v}{\sigma_{\widehat{X}_v}} \quad , \widehat{X}_v = \widehat{\mathrm{CL}_v},\ \widehat{\mathrm{RVI}_v},\ \widehat{\mathrm{LAI}_v},\ \widehat{\mathrm{DIFN}_v},\ \widehat{\mathrm{MTA}_v} \tag{8}$$

where $\sigma_{\widehat{X}_v}$ describes the standard deviation of $\widehat{X}_v$

To test for co-linearity among the growth traits, the variance inflation factor (VIF; see e.g. Belsley *et al.* (1980)) was calculated as

$$VIF(\widehat{X}_v) = \frac{1}{1-R^2} \tag{9}$$

where $VIF(\widehat{X}_v)$ is the variance inflation factor for the growth trait $\widehat{X}_v$. $R^2$ is the coefficient of determination for a model, in which the given growth parameter is the dependent variable and the remaining four growth traits are the independent variables. If *VIF* exceeded 5.0 for one or more of the measurements, the co-linearity between the growth traits was judged to be too strong; the variable with the greatest *VIF* was excluded from further analysis, and a new *VIF* for the remaining measurements was estimated. The analyses showed that DIFN should be excluded from the model (Results; Table 4).

**Table 4** Minimum, maximum, mean and standard deviation of the growth traits, $R^2$ from analyses where the mentioned growth traits were explained by the other traits and results from the analysis of variance inflation factor, VIF

| | | | | | Initial analysis | | DIFN excluded | |
|---|---|---|---|---|---|---|---|---|
| Growth char. | Min | Max | Mean | Std dev. | $R^2$ | VIF | $R^2$ | VIF |
| CL, cm | 54.90 | 82.50 | 64.95 | 0.59 | 0.37 | 1.6 | 0.37 | 1.6 |
| MTA, ° | 47.26 | 58.72 | 54.14 | 2.03 | 0.53 | 2.1 | 0.47 | 1.9 |
| LAI, $m^2m^{-2}$ | 4.24 | 5.57 | 4.86 | 0.27 | 0.79 | 4.8 | 0.35 | 1.5 |
| DIFN | 0.013 | 0.046 | 0.029 | 0.007 | 0.83 | 6.0 | | |
| RVI | 7.75 | 10.06 | 9.01 | 0.43 | 0.24 | 1.3 | 0.14 | 1.2 |

### *Combining results from both types of experiments*

Data from the two types of experiments were combined, to test if it was possible to use the variety-specific growth traits $\left(\widehat{CL_v},\ \widehat{RVI_v},\ \widehat{LAI_v},\ \widehat{MTA_v}\right)$ from the experiments without weeds (eqn. (8)) to predict weed coverage $(\widehat{C}_{vly})$ of the same varieties grown under organic conditions with some weeds present (eqn. (2)). We applied the model:

$$\widehat{C}_{vly} = \widehat{C}_{\max,ly}\begin{pmatrix} 1-\alpha-\beta_1\widehat{CL_v}-\beta_2\widehat{RVI_v}-\beta_3\widehat{MTA_v}-\beta_4\widehat{LAI_v} \\ -\gamma_1\widehat{CL_v}\widehat{RVI_v}-\gamma_2\widehat{CL_v}\widehat{MTA_v}-\gamma_3\widehat{CL_v}\widehat{LAI_v} \\ -\gamma_4\widehat{RVI_v}\widehat{MTA_v}-\gamma_5\widehat{RVI_v}\widehat{LAI_v}-\gamma_6\widehat{MTA_v}\widehat{LAI_v} \end{pmatrix} + D_{vly} + E''_{vly} \tag{10}$$

where $D_{vly}$ and $E''_{vly}$ are the interactions between varieties, locations and years and residual variation, respectively. As those two sources of variation cannot be separated here only the sum is estimated in the model. The sum is assumed to be normally distributed with zero mean and constant variance. The terms $\alpha, \beta_1, \beta_2, \beta_3, \beta_4, \gamma_1, \gamma_2, \gamma_3, \gamma_4, \gamma_5, \gamma_6$ and $\sigma^2_{\mathrm{D}} + \sigma^2_{E''}$ are the parameters to be estimated. To stabilize the variance, the parameters were estimated after square-root transformation on both sides of eqn. (10).

Successive reduction of the model was performed by excluding the least significant effect from the model. Some restrictions were imposed during the reduction process: The main effect of $\widehat{CL_v}$, $\widehat{RVI_v}$, $\widehat{LAI_v}$, or $\widehat{MTA_v}$ was not removed until all interactions with these variables were excluded from the model. When all remaining effects were significant at the 5% level the reduction was stopped.

After excluding non significant effects from the model, the final model was

$$\sqrt{\widehat{C}_{vly}} = \sqrt{\widehat{C}_{\max,ly}\begin{pmatrix} 1-\alpha-\beta_1\widehat{CL_v}-\beta_2\widehat{RVI_v}-\beta_3\widehat{MTA_v}-\beta_4\widehat{LAI_v} \\ -\gamma_3\widehat{CL_v}\widehat{LAI_v}-\gamma_4\widehat{RVI_v}\widehat{MTA_v} \end{pmatrix}} + D_{vly} + E''_{vly} \tag{11}$$

We calculated the varietal suppression index as

$$\widehat{\lambda}^*_v = \alpha + \widehat{\beta}_1\widehat{CL_v} + \widehat{\beta}_2\widehat{RVI_v} + \widehat{\beta}_3\widehat{MTA_v} + \widehat{\beta}_4\widehat{LAI_v} + \widehat{\gamma}_3\widehat{CL_v}\widehat{LAI_v} + \widehat{\gamma}_4\widehat{RVI_v}\widehat{MTA_v} \tag{12}$$

where $\widehat{\lambda}^*_v$ is the suppression index, and $\alpha, \widehat{\beta}_1, \widehat{\beta}_2, \widehat{\beta}_3, \widehat{\beta}_4, \widehat{\gamma}_3, \widehat{\gamma}_4$ are the parameters from eqn. (11), which were estimated by the method of maximum likelihood. To validate the model, the parameter $\widehat{\lambda^*_v}$ based on eqn. (12) was used to predict weed coverages recorded in the two field trials at Foulum and Dalmose in 2005. All statistical analyses were performed using the procedures MIXED, NLMIXED or CORR (SAS Institute Inc., 1999).

## Results

### *Weed coverage assessments*

The varieties showed a strong significant effect on weed coverage (adjusted for experimental design; $p<0.0001$). However, the largest variation was among environments (location*year; data not shown). The average weed coverage among the nine environments was 22%, ranging from 9% in Foulum 2002 to 43% at Flakkebjerg in 2004. The 90% quantile of weed coverage at plot level, ranged between 13% in Foulum 2002 and 61% at Flakkebjerg in 2004 (Table 2).

The model (eqn. (5)) estimated a wide range of suppressive indices ($\hat{\lambda}_v$) among the varieties, from the weakest suppressive varieties Granta and Ceylon at 0% to the strong suppressors Pallas and Modena at 57% (Fig. 1). The model was able to explain between 13% (Jyndevad 2004) and 77% (Flakkebjerg 2004) of the variation in weed coverage within the environments (Fig. 2).

**Table 5** Estimated parameters and significance levels for culm length (CL), relative vegetation index (RVI), mean tilt angle (MTA), leaf area index (LAI) and the two significant interactions. The parameters were estimated using eqn. (11)

| Parameter | Estimate | Standard error | t-value | p>\|t\| |
|---|---|---|---|---|
| $\alpha$ | 0.293 | 0.009 | 33.87 | <0.0001 |
| $\beta_1$ *(CL)* | 0.063 | 0.010 | 6.09 | <0.0001 |
| $\beta_2$*(RVI)* | 0.030 | 0.009 | 3.30 | 0.0010 |
| $\beta_3$*(MTA)* | -0.010 | 0.013 | -0.80 | 0.4262 |
| $\beta_4$*(LAI)* | 0.009 | 0.011 | 0.83 | 0.4069 |
| $\gamma_3$*(CL× LAI)* | -0.038 | 0.010 | -3.92 | <0.0001 |
| $\gamma_4$*(RVI×MTA)* | -0.020 | 0.010 | -2.09 | 0.0371 |

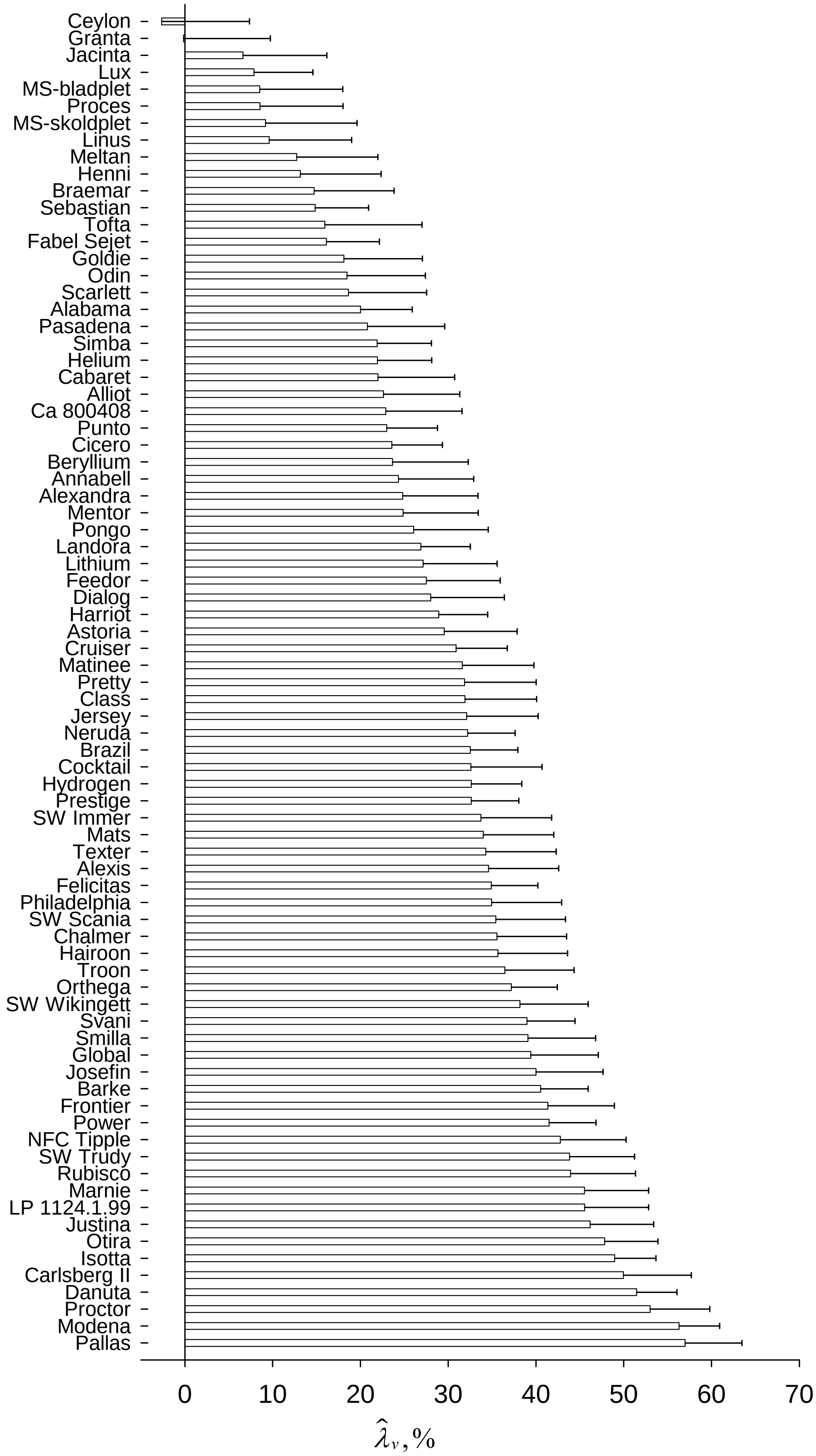


**Fig. 1** Estimated relative suppression of weed coverage for 79 spring barley varieties from eqn (5). Horizontal lines indicate standard error of the estimated values**.**

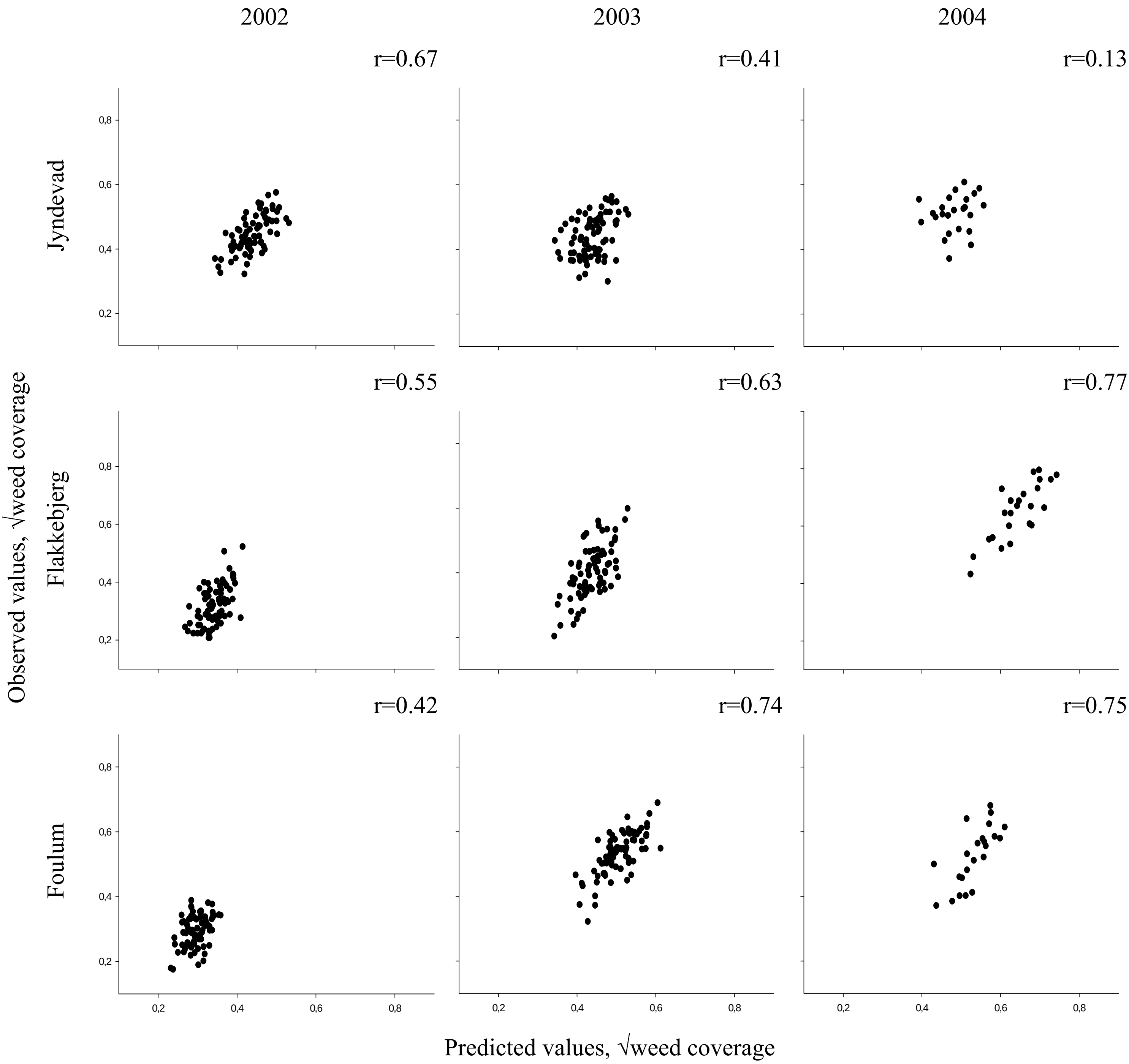


**Fig. 2** Relationship between square-root transformed predicted values from eqn. (5) and observed values for weed coverage at the three locations in 2002, 2003 and 2004. Correlation coefficients are given at the top right corner for each environment. Observed values are based on three replicates in 2002 and 2003 and two replicates in 2004.

### *Growth parameters*

There were strong significant differences among varieties ($p<0.0001$) for all the measured growth parameters of eqn. (8) (Table 4). The analysis of the variance inflation factor (VIF) showed that diffuse non-intercepted radiation (DIFN) and LAI were closely correlated as VIF was 6.0 for DIFN and 4.8 for LAI. By excluding DIFN in the next analysis, VIF of LAI was reduced to 1.5. As VIF for the other growth traits did not exceed 2.0, the traits used into the final analysis were RVI, CL, MTA and LAI (Table 4). A similar VIF analysis was conducted between all possible two-sided interactions of the four remaining growth traits. This analysis did not show any strong co-linearity between these interactions, therefore no two-sided interactions were excluded from further analysis due to co-linearity.

### *Combination of the data from the two types of experiments*

The two types of data were combined and initially analysed. After reduction the final model became eqn. (11). We found significant main effects of CL and RVI and found that LAI interacted significantly with CL, as did RVI with MTA. The correlations between predicted values from eqn. (11) and observed values (both square-root transformed) from the nine environments are shown in Fig. 3. The estimated parameters from eqn. (11) are shown in Table 5. Notice that the parameter from eqn. (11) for CL ($\beta_1$), RVI ($\beta_2$) and LAI ($\beta_4$, although not significant) were positive i.e. weed suppression increased with culm length, RVI or LAI. The parameter for MTA was negative, meaning that more erect leaves (greater MTA) reduced weed suppression. However, this parameter was not significantly different from zero. The significant interactions CL×LAI and RVI×MTA indicated that the main effects, CL or RVI depended on of the level of LAI or MTA, respectively. The parameter estimates of the interactions ($\gamma_3$ and $\gamma_4$) were both negative, meaning that the effect of increasing either CL or LAI would become reduced by an increase in the other. The ranking of the 79 varieties based on this model is shown in Fig. 4.

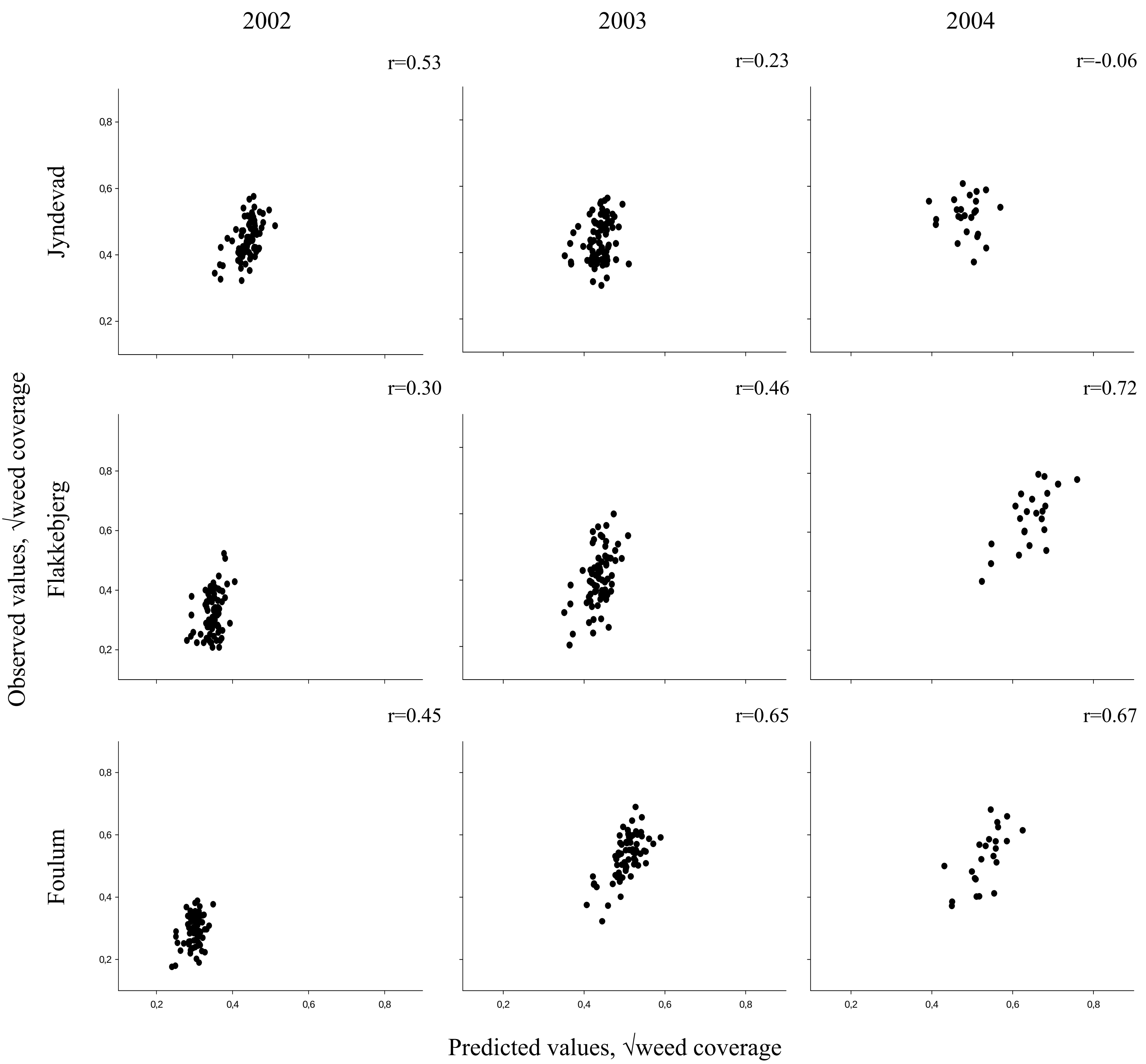


**Fig. 3** Relationship between square-root transformed predictions from eqn. (11) and observed values for weed coverage at the three locations in 2002, 2003 and 2004. Correlation coefficients are given at the top right corner for each environment. Observed values are based on three replicates in 2002 and 2003 and two replicates in 2004.

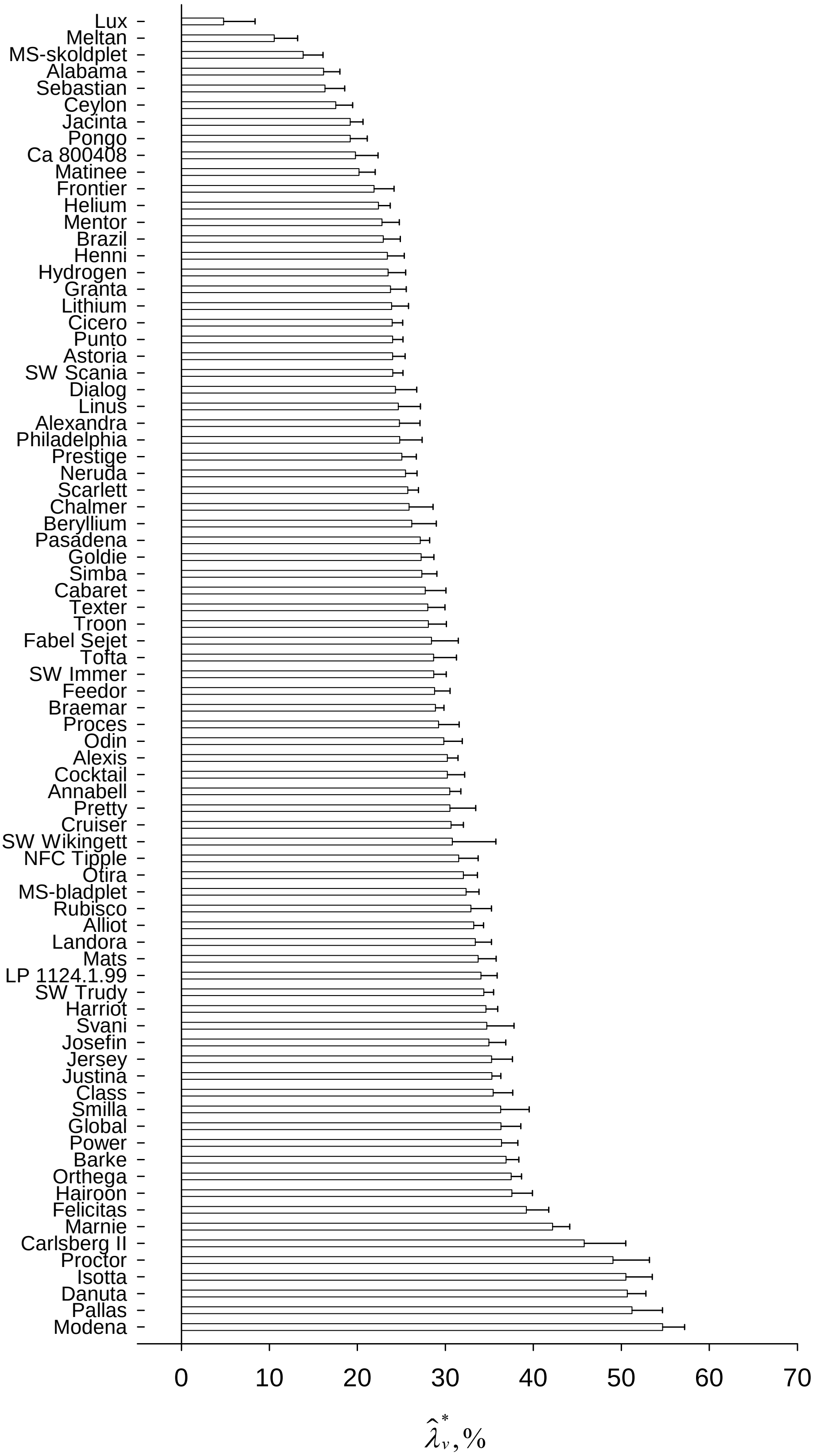


**Fig. 4** Estimated relative suppression of weed coverage for 79 spring barley varieties from eqn. (11) Horizontal lines indicate standard error of the estimated values.

The weed coverage predicted from eqn. (11) was compared to that obtained using eqn. (5) (Fig. 5, $R^2$=0.76). This yielded an underestimate for strong suppressors and an overestimate for weak suppressors in some environments (i.e. Jyndevad 2003 and 2004; Fig. 2).

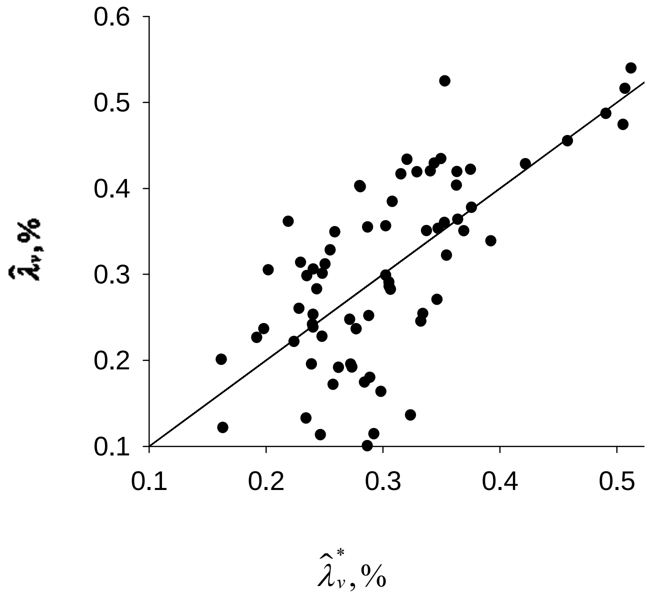


**Fig. 5** Correlation between predicted suppression indices from eqn. (5) $(\widehat{\lambda_v})$ and eqn. (11), $(\widehat{\lambda_v^*})$ for 79 varieties. $R^2$=0.76. Solid line indicate y=x.

An analysis showed a significant positive correlation between $\widehat{C}_{\max,ly}$ and the correlation coefficients between the predicted and the observed weed coverage (Fig. 6) for both eqn. (5) and eqn. (11) – with the environment Jyndevad 2004 being an exception. This indicated that the accuracy of the weed coverage predictions increased with increasing maximal weed coverage.

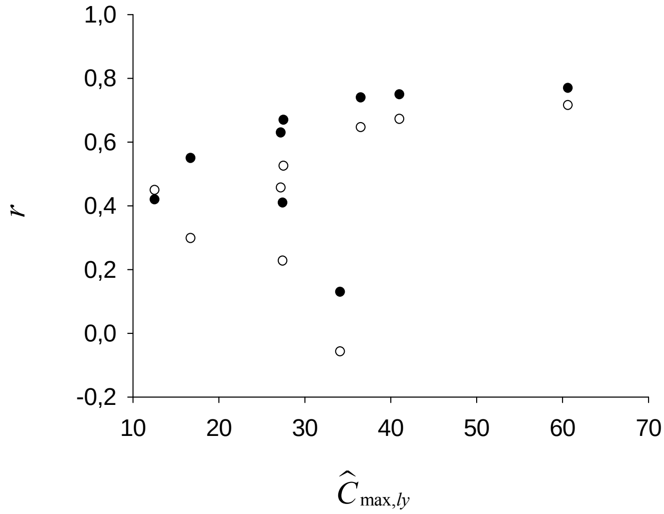


**Fig. 6** Correlation coefficient for eqn. (5) (●) and eqn. (11) (○) at different weed pressures $(\widehat{C}_{\max,ly})$.

### *Validation*

The validity of the suppressive index was tested at two locations in 2005. At Foulum the 90% weed coverage quantile, $\widehat{C}_{\max,ly}$ was 38%, while at Dalmose we observed very low weed infestations with a $\widehat{C}_{\max,ly}$ of only 11%. Weed coverage was underestimated at Foulum for varieties like Danuta and Orthega (Fig. 7), while it was overestimated for varieties like Isotta and Harriot. About 37% of the variation in weed coverages in this environment could be explained by the estimated suppression indices using eqn. (11). At Dalmose, only a weak correlation between predicted and observed weed coverage assessments was found ($R^2$ =0.25). Weed coverage was underestimated for Fabel Sejet, while an overestimation was observed for most of the other varieties particularly for varieties like Alabama and Svani.

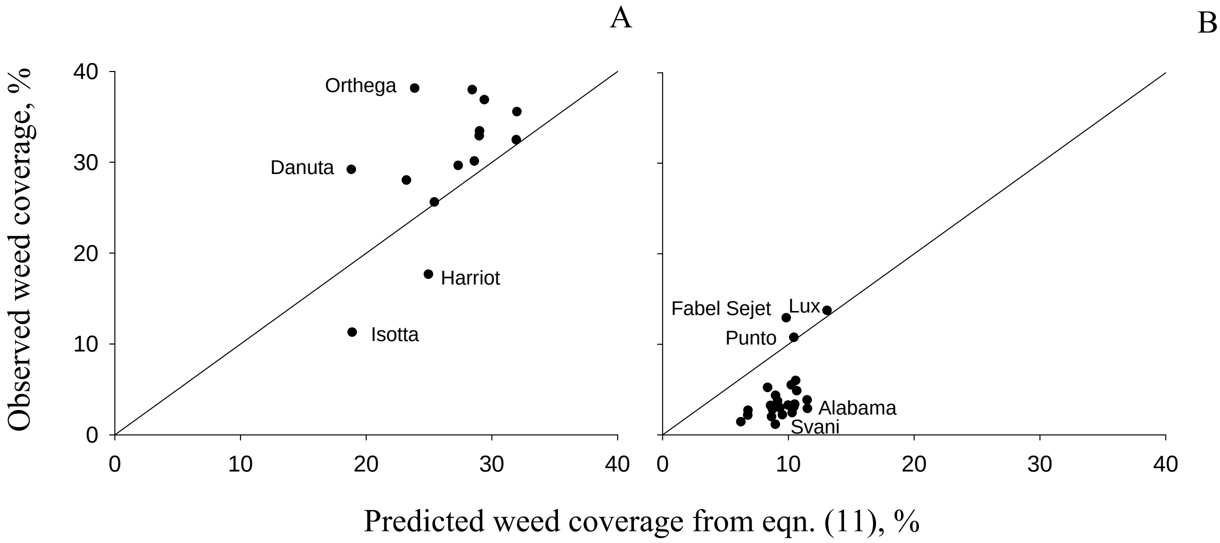


**Fig. 7** Validation of eqn. (11) at two locations. A) Foulum 2005 (r=0.61), and B) Dalmose 2005 (r=0.50). Solid line indicate y=x.

## Discussion

### *Weed coverage assessments*

The model (eqn. (5)), which estimated the weed suppressive ability from the assessed weed coverages, explained between 13 and 77% of the variation of the square-root transformed weed coverages. The lowest amount of explained variation was in Jyndevad 2004 with only 24 varieties. The poor prediction in this experiment was caused by a marked change in the ranking of the varietal suppressive ability, as one of the most suppressive varieties, Modena, had very poor weed suppression here (data not shown). By comparing Jyndevad 2003 with Jyndevad 2004 it was observed that some extreme varieties like Fabel Sejet and Neruda changed from a very strong to a weak suppressor and vice versa (data not shown). The Jyndevad location had a coarse sandy soil, which in all three years was sown earlier than Flakkebjerg and Foulum, and it was the only location where irrigation was used. These differences in site characteristics may have caused interactions that changed the rankings of the varieties as discussed by Cousens & Mokhtari (1998). Even though the estimated relative reduction for the least suppressive varieties, like Granta, Ceylon and Jacinta (Fig. 1), was not significantly different from 0, still a large reduction occurred compared to no crop at all. In fact, weeds just outside the plots were markedly greater than within plots.

### *Combining the two types of data*

The results showed that we were able to use data from one type of experiment to explain the weed coverage in another type of experiment with up to 72% explained variation. In comparison, within one experiment, Lemerle *et al.,* (1996) could explain 32% of the variation in weed suppression in spring wheat based on canopy height, tiller number and a score for leaf habitus. We underestimated the suppressive ability for strong suppressors and overestimated for weak suppressors but for the purpose of classification or ranking of varieties, this may be acceptable as a conservative method. Some varieties like Frontier and MS-bladplet showed marked differences in weed suppressive ability when estimated by the growth traits (Fig. 4) or from weed coverage (Fig. 1). This could be due to varietal interactions between below and above-ground growth (Wilson, 1988). For four varieties of winter wheat, De Lucas Bueno and Froud-Williams (1994) found that competition for below ground resources were greater than for above ground resources.

Verschwele and Niemann (1993) found crop coverage, canopy height and growth rate to be important for characterisation of the shading ability in winter wheat and they combined the three growth parameters into scores from visual assessments of the variety. In our study crop growth dynamics were summarised by measuring reflectance in an early growth stage (RVI), leaf area index (LAI) and mean tilt angle (MTA) in mid-season, and culm length (CL) at the end of the growing season. Such machine measurable growth traits have the advantage of being open to standardisation. Christensen (1995) obtained a good description of varietal differences in weed suppressive ability using an additive model including maximum canopy height, maximum light interception and temporal displacement of the light interception. We found significant effects of CL and RVI but also interactions between CL and LAI and between RVI and MTA, Thus suppressive ability cannot always be attributed to additive effects only, as our results indicated that the growth traits interact.

We found strong suppressive varieties primarily among older varieties, characterised by long culm lengths and planophile leaves: e.g. Proctor, Pallas and Carlsberg II which were bred in the mid 1960's. In the last two to three decades this ideotype was deselected by plant breeders due to high risk of lodging at the high nutrient levels and undesirable high production of straw. At that time breeding aimed at creating short varieties with erectophile leaves with low risk of lodging. However, in the last decade, increasing production of straw for energy use, reduced nutrient quotas and increasing pressure to reduce herbicide use have made some Danish companies interested in breeding strongly competitive varieties.

Variety choice is often part of the growing strategy under organic or low-input growing conditions. Drews *et al*. (2002) developed strategies to increase competitiveness of wheat cultivars through shading. They found that variety and row width affected ground coverage and light interception and influenced weed growth. With narrow row distance (12 cm) and the same plant density (400 seeds per $m^2$), varieties with erect leaves suppressed weeds as well as varieties with planophile leaves. However with greater row distance (24 cm), varieties with a planophile leaf structure were more competitive since they achieved a better ground coverage. These findings were supported by Eisele and Köbke (1997a; 1997b), who concluded that by using the right combination of row width, planting direction and variety, growing the crop without use of mechanical weed control might be possible under low weed infestation levels.

The introduction of a robust index for weed suppression will make two choices possible: a variety for reduction in weed seed production for long term strategic reasons (Lemerle *et al.,* 1996) or a variety with weak suppressive ability for use as a cover crop to improve establishment and development of the under sown crop. The results of this study show that it is possible to use just four measurements of growth traits under weed-free conditions to predict weed coverage under normal field conditions and to estimate a weed suppression index that corresponds well to observed suppressive ability.

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# Paper I. Tolerance of four spring barley (*Hordeum vulgare* L.) varieties to weed harrowing

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## Summary

We investigated the tolerance to weed harrowing of four spring barley varieties and examined the possible interactions between varietal weed suppressive ability and nutrient level. Tolerance was defined as the combined effect of crop resistance (ability to resist soil covering) and crop recovery (the ability to recover in terms of yield). The weed harrowing strategy was a combination of one pre- and one post-emergence weed harrowing.

In terms of yield, the four varieties responded significantly different to weed harrowing, and the response depended on nutrient level. At the lower nutrient level, weed harrowing caused an increase in yield of 4.4 hkg ha$^{-1}$ for a strong competitor (*cv.* Otira), while there was no effect on yield at the higher nutrient level. For a weaker competitor (*cv.* Brazil), weed harrowing caused no change in yield at the lower nutrient level, whereas yield decreased by 6.0 hkg ha$^{-1}$ at the higher nutrient level. There were marked differences between the weed suppressive ability of the four varieties when not harrowed, with less pronounced but significant

differences when harrowed. Weed harrowing did not change the weed suppressive ability of a variety.

**Keywords:** mechanical weed control, cereal varieties, weed competition, image analysis, spring barley cultivars.

**Total words:** 6665

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## Introduction

Weed management in organic or low-input growing systems relies on the integration of preventive and curative methods (Barberi, 2002). Preventive methods like crop rotation (Bond & Grundy, 2001), fertiliser placement (Rasmussen, 2002) and use of competitive species and varieties (Lemerle *et al.,* 2001) can keep weed populations at a manageable level within the growing system as a whole, while curative methods like pre- and post-emergence weed harrowing (Rasmussen, 1991) are required to control weeds when thresholds are exceeded. The spring tines of the harrow control weeds by uprooting and/or covering small weed plants with soil (Kurstjens & Kropff, 2001).

Pre- and post-emergence weed harrowing is often used in combination in organically grown spring cereals. Timing is important for the success of pre-emergence weed harrowing, because it should be conducted just before crop emergence to ensure effective weed control without harming the crop (Rasmussen & Rasmussen, 1999). The efficacy of post-emergence weed harrowing relies on its selectivity, which has been defined as the ratio between the positive weed control effect and the negative crop cover effect (Rasmussen, 1992). If the weed plants are large relative to the crop plants selectivity is reduced, and the risk of damaging the crop mechanically or by soil coverage is increased (Rasmussen, 1991). The risk of crop damage also rises with the intensity of weed control, which is determined by the speed or aggresivity of the spring tines (Kurstjens & Kropff, 2001). Crop damage due to weed harrowing has been shown to reduce yield (Kirkland, 1994; Rasmussen & Svenningsen, 1995; Jensen *et al.,* 2004). Apart from the direct effect on yield through changes in crop growth, indirect effects on crop-weed competition from altered conditions may be important.

*Tolerance* to weed harrowing has been defined as the combined characteristics of the crop to *resist* initial damage caused by weed harrowing and to *recover* from this damage (Gundersen *et al.,* 2006). Resistance to initial damage is related to the height of the crop and the flexibility and shape of the leaves (Kurstjens & Perdok, 2000). Kurstjens and Kropff (2001) found that uprooting was important for the resistance of the crops, *Lolium perenne* L., *Lepidium sativum* L. and *Chenopodium quinoa* Willd. However, for strongly-anchored plants, like cereals, soil covering is likely more important than uprooting. A crop with high *recovery* is characterised by growth traits well-suited to overcome soil covering and maintain yield. The degree of recovery from soil covering depends on burial depth, soil texture and plant recovery processes (Baerveldt & Ascard, 1999; Kurstjens & Kropff, 2001).

Lemerle *et al.* (2001) describe several studies showing strong varietal differences in weed suppression. The majority of these studies have been conducted as a comparison between weedy and weed-free (herbicide treated) conditions. Only a few studies have been conducted to estimate varietal differences in response to weed harrowing in cereals and to study if weed harrowing interacts with weed suppressive ability. Rasmussen *et al.* (2004) measured tolerance in spring barley (*Hordeum vulgare* L.) as the relative reduction in yield and found tolerance was negatively correlated with growth traits associated with weed suppressive ability.

The aim of this study was to investigate the tolerance of four spring barley varieties to weed harrowing under organic growing conditions at two nutrient levels. The weed harrowing strategy was a combination of one pre- and one post-emergence weed harrowing as described by Rasmussen and Rasmussen (1995). We estimated the effect of weed harrowing (1) on soil covering of the crop just after harrowing, (2) on yield and (3) investigated the possible interactions among variety, weed harrowing and weed suppression.

## Materials and methods

### *Experimental conditions*

Four varieties of spring barley were chosen to represent the range in varietal weed suppressiveness among varieties in the Danish variety list (Anon, 2005c). The weed suppressive index (*SI*) of the varieties was for Modena (0.75), Orthega (0.91), Otira (0.98) and Brazil (1.04) (Hansen *et al.,* 2006). *SI* indicates the expected relative amount of weed cover, where 1.00

equals an average variety. Suppressive varieties have lower SI values, and Modena was expected to be the most suppressive variety and Brazil the least.

The varieties were studied in field trials at Research Centre Flakkebjerg (55°19'N, 11°24'E) in 2004 and 2005 on sandy loam containing 12.4% clay, 60.1% silt, 25.5% sand and 2.0% organic matter. In 2004 and 2005 the precipitation from sowing to harvest was 283 mm and 207 mm, respectively. Despite the greater precipitation in 2004, spring was characterised as being drier than in 2005. Growing degree-days (d °C), accumulated from the date of sowing with a base temperature of 0 ºC, was used. The interval from sowing to harvest was 1724 d °C in 2004 and 1660 d °C in 2005. The crop rotation of the experimental areas is shown in Table 1. The soil was mouldboard ploughed to a depth of 25 cm in late autumn.

**Table 1** Crop rotation in experimental fields prior to experiment

| Year | 2004 | 2005 |
|---|---|---|
| 2000 | Oats (*Avena sativa* L.) | |
| 2001 | Spring barley with white clover (*Trifolium repens* L.) under sown | Lucerne (*Medicago sativa* L.) |
| 2002 | White clover for seed production | Lucerne |
| 2003 | Winter rape (*Brassica napus ssp. napus* L.) | Oats |
| 2004 | Spring barley, experiment | Winter wheat (*Triticum aestivum* L.) |
| 2005 | | Spring barley, experiment |

The field trials were split-plot designs. Whole plots consisted of the eight combinations of three factors; two levels each of herbicides (±), weed harrowing (±), and nutrient level (40% or 80% of the recommended nitrogen need) (Anon, 2003). The eight subplots were arranged in two neighbouring rows with four subplots per row. Each subplot consisted of the four varieties in pure stands, three two-component mixtures and one three-component mixture of the varieties. The mixtures were not considered but were included in the primary statistical analysis to adjust for experimental design. An α-design was used to optimise the comparisons between varieties within whole plots (Patterson & Williams, 1976). With three replicates, there were 192 plots each year.

The gross plot size was 2.5×14.5 $m^2$ and the net plot size was 1.50×12.0 $m^2$. The net plots were split into a part used for non-destructive measurements and combine harvesting (1.5×9.5 $m^2$) and a part used for destructive measurements (1.5×2.5 $m^2$).

The crop was sown with a seed drill with 12.0 cm row width on 15 April 2004 and 13 April 2005. Seed rates were adjusted for seed weights and germination rate to give a population of 350 plants $m^{-2}$. As model weeds we used a mixture of 25% viable seeds of *Chenopodium album* L., 25% *Phaselia tanacetifolia* Benth*.,* 25% *Brassica napus ssp. napus* L. and 25% *Trifolium incarnatum* L. *cv.* Poppelsdorfer in plots with no pesticide treatments. The weeds were sown 16 April 2004 and 13 April 2005 at a density of 200 seeds $m^{-2}$. The naturally occurring weeds were *Stellaria media* (L.) Vill., *Sinapsis arvensis* L., *Viola arvensis* Murray*, Verónica arvensis* L.*, Thlaspi arvense* L. and *Polygonum convolvulus* L.. The total density of these species did not exceed 50 plants $m^{-2}$, and the biomass of these species was included in the total weed biomass. Due to heterogeneous infestations of *Cirsium arvense* (L.) Scop. in the experiments, the density of this species was recorded at 6 July 2004 (992 d °C) and 1 August 2005 (1435 d °C).

In the herbicide-treated plots, we applied a mixture of 7.5 g tribenuron-methyl $ha^{-1}$ (Express ST, 500 g a.i $kg^{-1}$, DuPont), 108 g fluroxypyr $ha^{-1}$ (Starane 180, 180g a.i. $l^{-1}$ Dow AgroSciences) and 150 g surfactant $ha^{-1}$ (Lissapol Bio, 1000 g a.i. $l^{-1}$, Syngenta Crop Protection) at 12 May 2004 (310 d °C). In 2005 (13 May 2005, 265 d °C) we applied a mixture of 24 g ioxynil + 24 g bromoxynil $ha^{-1}$ (Oxitril CM, 200g + 200g a.i. $l^{-1}$, Bayer CropScience), 0.0255 g mefenpyr-diethyl $ha^{-1}$ + 0.0085 g iodosulfuron-methyl-Na $ha^{-1}$ (Hussar, 150g a.i.$kg^{-1}$ + 50g a.i.$kg^{-1}$, Bayer CropScience) + 400 g surfactant $ha^{-1}$ (Isoblette, 1000 g a.i. $l^{-1}$, Bayer CropScience). The applications were performed at a dosage of 150 l $ha^{-1}$ with nozzle type S-ISO-LD-02-110 (Hardi International, Helgeshøj Allé 38, Taastrup, Denmark) and a pressure of 230 kPa. Driving speed was 6 km $h^{-1}$. All dosages and mixtures were determined using the decision support system Crop Protection Online (Anon, 2005b). We assumed no interactions between herbicide treatments and the growth and development of the varieties.

One pre-emergence weed harrowing was conducted on 25 April 2004 (129 d °C) and 21 April 2005 (79 d °C). The driving speed was approximately 9 km $h^{-1}$. On 13 May 2004 (319 d °C, crop growth stage (GS, Lancashire *et al.,* 1991) 21-22) and 17 May 2005 (300 d °C, crop GS 21-25) one post-emergence weed harrowing was conducted with a driving speed of approximately 7-8 km $h^{-1}$. The weeds were between cotyledon stage to four true leaves. The intensity of harrowing was adjusted by driving speed in an attempt not to exceed 20% crop burial at the post-emergence weed harrowing as an average. Weed harrowing was carried out with a spring-tine harrow (Einböck, Dorf an der Pram, Austria). The post-emergence weed harrowing in 2004 was done on humid soil, while the soil was dry on the surface in 2005. In both years pre- and the post-emergence weed harrowing were conducted under sunny and windy conditions resulting in fast drying of the soil.

### *Measurements*

Tolerance to weed harrowing was measured as an immediate effect (area of plants covered with soil just after weed harrowing) and a long-term effect (yield). To estimate the degree of soil cover, two digital images were acquired twice weekly in every plot from crop emergence until three weeks after the post-emergence weed harrowing; subsequently images were acquired weekly. Extra photos were acquired immediately prior to the post-emergence weed harrowing. We used a Canon PowerShot G1 Camera. The exact positions of the images in the plots were marked to ensure that images were acquired at the same spot every time. The camera was mounted on a stand covered with white sheet clothing, to provide diffuse lighting conditions and to eliminate shadows and highlighted areas. The camera height was approximately 133 cm above the soil surface. The resolution of the images was 2086×1548 pixels, and they covered approximately 450×350 mm on the soil surface. Thus each pixel covered 0.22×0.22 mm soil surface. The camera set focus, ISO speed, white balance and shutter speed automatically. Images were saved as Canon RAW format, and converted to 24-bit PPM format with the free-ware program DCRAW.EXE (Anon, 2005a). Images were loaded into MatLab 6.5 (Anon, 2002) as RGB-images and were converted to 8-bit greyscale images to make the green pixels more pronounced by using a slightly modified version of the algorithm described by Woebbecke *et al.* (1995):

$$g_{x,y} = 2G_{x,y} - R_{x,y} - B_{x,y} \tag{1}$$

where $g_{x,y}$ is the greyscale value of a pixel at position $(x, y)$ in the image. $R$, $G$ and $B$ are non-normalised values from the red, green and blue channel, respectively. To segment the pixels with high intensity (former green) from pixels with low intensity (former non-green), a modified version of an automatic thresholding technique was used, which chose the threshold to minimize the intra-class variance between green and non-green pixels (Otsu, 1979). After thresholding, a median filter was applied to reduce “salt-and-pepper noise.” Vegetation cover ($VC$; %) was estimated in every image as the relation between the number of vegetation pixels and the total number of pixels in the binary images. Weed harrowing covered the leaves with soil, which was measured as the difference between *VC* just before and just after harrowing ($\Delta VC$; percentage point).

Canopy height (*H*; cm) was measured in the same positions as the images in the herbicide-treated, non-harrowed plots at 19 May 2004 (386 d °C) and 25 May 2005 (396 d °C) with a circular plate divided into four quarters. The plate had an area of 0.25 $m^2$ and was mounted on a measuring stick. The canopy height was defined as the vertical distance from soil surface to the underside of the plate when at least one leaf touched each of the four quarters of the plate.

Leaf area index (*LAI*; $m^2$ leaf area per $m^2$ ground area) was measured 8 June 2004 (613 d °C) and 1 June 2005 (496 d °C). We measured twice in the same positions as the images and canopy heights, using LICOR 2000 Canopy Analyzer (Lang *et al.,* 1985; Welles & Norman, 1991).

Weed biomass ($DM_W$; g $m^{-2}$) was measured at 11 June 2004 (659 d °C, crop GS 41-43) and 15 June 2005 (659 d °C, crop GS 41-49) in all plots by cutting the plant material at the soil surface in a square 0.25 m² frame. The plant material was separated into crop and weeds. The samples were dried at 100°C for 24 hours and dry matter was measured. The interval in d °C between post-emergence weed harrowing and biomass measurement was (659-319=340 d °C) in 2004 and (659-300=359 d °C) in 2005. The experiments were harvested at 19 August 2004 and 16 August 2005 with a combine plot harvester, and the yield, (*Y*; hkg $ha^{-1}$) was adjusted to 85% dry matter.

Crop density ($D_C$; plants $m^{-2}$) was recorded before post-emergence harrowing on 4 May 2004 (207 d °C) and 11 May 2005 (247 d °C) as the number of crop plants in one-meter crop row replicated three times randomly in every plot.

### *Statistics*

To adjust for experimental design and inhomogeneous presence of *C. arvense*, $\Delta VC$, $D_C$, $DM_W$ and *Y* were analysed with following model

$$\begin{aligned} X_{grcnhmv} &= \mu + \alpha_n + \beta_h + \gamma_m + \delta_v + [\text{all 2-factor interactions}] + \\ &[\text{all 3-factor interactions}] + [\text{the 4-factor interaction}] + Jt_{grcnhmv} + \\ &E_g + F_{gnhm} + G_{gr} + H_{gc} + I_{grcnhmv} \end{aligned} \tag{2}$$

where $X_{grcnhmv}$ is the response ($\Delta VC$, $D_C$, $DM_W$, *LAI* and *Y*) recorded for variety *v* (regarding each variety mixture as a 'variety'), in replicate *g* and treated with nutrient level *n*, herbicide level $h$ and weed harrowing level $m$ (and located in row $r$ and column $c$). $Jt_{grcnhmv}$ is the density of *C. arvense,* which was considered as random covariate. $E_g$ is the random effect of replicate $g$. $F_{gnhm}$ is the random effect of the whole plot with treatment combination $nhm$ in replicate $g$. $G_{gr}$ is the random effect of the incomplete block $r$ (in the α-design) in replicate $g$. $H_{gc}$ is the random effect of column $c$ in replicate $g$, and $I_{grchmv}$ is the residual variance, which is considered randomly distributed. We assumed all random effects to be normally distributed with mean zero and constant variances: $\sigma_E^2, \sigma_F^2, \sigma_G^2, \sigma_H^2, \sigma_I^2$. Greek letters indicate systematic effects. The two years were analysed individually and statistical analyses were done by the maximum likelihood method in the mixed linear model procedure (PROC MIXED) (SAS Institute Inc., 1999). To ensure variance stability, $VC$ was logit transformed and $DM_W$ was square root transformed. For $LAI$, $Y$ and $\Delta VC$ no transformation was necessary. Based on the model parameters we estimated yield, change in vegetation cover, $LAI$, crop density and weed biomass for each plot by the following model:

$$\begin{aligned}\widehat{X}_{nhmvg} &= \hat{\mu} + \hat{\alpha}_n + \hat{\beta}_h + \hat{\gamma}_m + \hat{\delta}_v + [\text{all 2-factor interactions}] + \\ &[\text{all 3-factor interactions}] + [\text{the 4-factor interaction}] + \hat{I}_{grcnhmv}\end{aligned} \tag{3}$$

where $\widehat{X}_{nhmvg}$ is the response ($\hat{Y}_{nhmvg}$, $\widehat{DM}_{Wnhmvg}$, $\widehat{\Delta VC}_{nhmvg}$, $\widehat{LAI}_{nhmvg}$ $\widehat{D}_{Cnhmvg}$) for each treatment and replicate adjusted for experimental design and presence of *C. arvense*. $\hat{\mu}$, $\hat{\alpha}_n$, $\hat{\beta}_h$, $\hat{\gamma}_m$, $\hat{\delta}_v$ and $\hat{I}_{grcnhmv}$ indicate the estimated parameter values for $\mu, \alpha_n, \beta_h, \gamma_m, \delta_v$ and $I_{grchmv}$ from eqn. (2). We excluded all data from variety mixtures and used the estimates in all further analyses.

The effect of weed harrowing in combination with the varietal weed suppressive ability on the weed biomass was estimated by

$$\Delta W_{nvg} = \widehat{DM}_{W\,nh-m+vg} - \widehat{DM}_{W\,nh-m-vg} \tag{4}$$

where $\Delta W_{nvg}$ corresponds to the absolute reduction in weed biomass after harrowing in replicate $g$ and variety $v$ at nutrient level $n$, $h$- indicates herbicide untreated plots, $m$+ indicates weed harrowed plots and $m$- indicates mechanically untreated plots. The effect of mechanical weed control on yield were estimated by

$$\Delta Y_{nvg} = \widehat{Y}_{nh+m+vg} - \widehat{Y}_{nh+m-vg} \tag{5}$$

where $\Delta Y_{nvg}$ corresponds to the absolute yield reduction due to harrowing, *h*+ indicates herbicide treated plots (assuming no influence from weeds), and the other indices are as described above.

As there were significant treatment effects on crop density after pre-emergence harrowing, we estimated the difference in crop density between pre-emergence harrowed and non-harrowed plots by the following model:

$$\Delta D_{C\,nvg} = \widehat{D_C}_{\,nhm+vg} - \widehat{D_C}_{\,nhm-vg} \tag{6}$$

where $\Delta D_{C\,nvg}$ indicates the difference in crop density due to pre-emergence harrowing, *m*+ indicates harrowed plots, *m*- indicates non-harrowed plots, and the other indices are as described above. These estimated values were used in the following analysis by a mixed linear model, which was common for the two years:

$$\Delta X_{nvyg} = \mu' + \alpha'_n + \delta'_v + \phi_y + \varphi_{nv} + \eta_{ny} + \iota_{vy} + \kappa_{nvy} + K_{nvyg} \tag{7}$$

where $\Delta X_{nvg}$ is the response (either $\Delta Y_{nvg}$, $\Delta W_{nvg}$, $\Delta VC_{nvg}$ or $LAI_{nvg}$) and $\alpha'_n$ is the effect of nutrient level, $\delta'_v$ is the effect of variety, $\phi_y$ is the effect of year, $\varphi_{nv}$ is the interaction between variety and nutrient level, $\eta_{ny}$ is the interaction between nutrient level and year, $\iota_{vy}$ is the interaction between variety and year, $\kappa_{nvy}$ is the three-way interaction and $K_{nvgy}$ is the residual variance which is assumed random and normally distributed with a constant variance of $\sigma_K^2$.

Finally we analysed if $\Delta VC$ or $\Delta D$ could explain the varietal differences in weed biomass and yield. We used data from plots with mechanical weed control but without herbicide treatment and analysed it with the following model:

$$\widehat{X}_{nvgm+h-y} = \mu^* + \alpha_n^* + \beta_y^* + \chi_{ny}^* + \delta^*(Z)_{nvgm+h-y} + E^*_{nvgm+h-y} \tag{8}$$

where $\widehat{X}_{nvgm+h-y}$ is the response variable (either $\widehat{W}_{nvgm+h-y}$ or $\widehat{Y}_{nvgm+h-y}$), $\alpha^*$ is the effect of nutrient level, $\beta^*$ is the effect of year, $\chi^*$ is the effect of the interaction between nutrient level and year $\delta^*$ is the effect of Z, (either $\Delta VC$ or $\Delta D$ ) used as a covariate, and $E^*_{nvgm+h-y}$ is the error which is considered random and normally distributed.

## Results

Crop density ($D_C$) was measured before post-emergence weed harrowing and could thus only be affected by the pre-emergence harrowing. Measurements conducted after post-emergence harrowing: weed biomass ($DM_W/\Delta W$), change in vegetation cover ($\Delta VC$) and yield ($Y/\Delta Y$), reflect the combined effect of both harrowings. Vegetation cover (*VC*) was measured both after pre- and post-emergence harrowing.

The varieties differed significantly in canopy height with similar patterns in both years, Modena and Brazil achieving the largest and smallest final height, respectively (Table 2).

**Table 2** Canopy height (H; one week after post-emergence harrowing and final) and leaf area index (LAI; final). Average (standard error) of both nutrient levels

| | Canopy height; *H* (cm) | | | | LAI | |
|---|---|---|---|---|---|---|
| | 2004 | | 2005 | | | |
| Variety | 19 May | Final | 24 May | Final | 2004 | 2005 |
| Modena | 17 (0.5) | 92 (1.3) | 20 (0.9) | 97 (1.7) | 4.1 (0.1) | 3.4 (0.1) |
| Otira | 14 (0.6) | 81 (1.3) | 15 (0.9) | 79 (1.8) | 4.7 (0.1) | 3.4 (0.1) |
| Orthega | 18 (0.5) | 79 (1.3) | 21 (0.9) | 82 (1.7) | 4.2 (0.1) | 3.1 (0.1) |
| Brazil | 14 (0.6) | 76 (1.3) | 13 (0.9) | 74 (1.8) | 3.9 (0.1) | 2.4 (0.1) |

### *Effect of pre-emergence weed harrowing on vegetation cover and density*

Pre-emergence weed-harrowing reduced vegetation cover (*VC*; measured just before post-emergence weed harrowing) at the high nutrient level in herbicide-treated plots (p=0.0165), from 39% in the non-harrowed to 35% in the harrowed plots (average of the two years), while the low nutrient treatment had an average of 34%, irrespective of weed harrowing (Fig. 1). There was a strong additive effect of variety on *VC*, as variety did not interact with the other treatments.

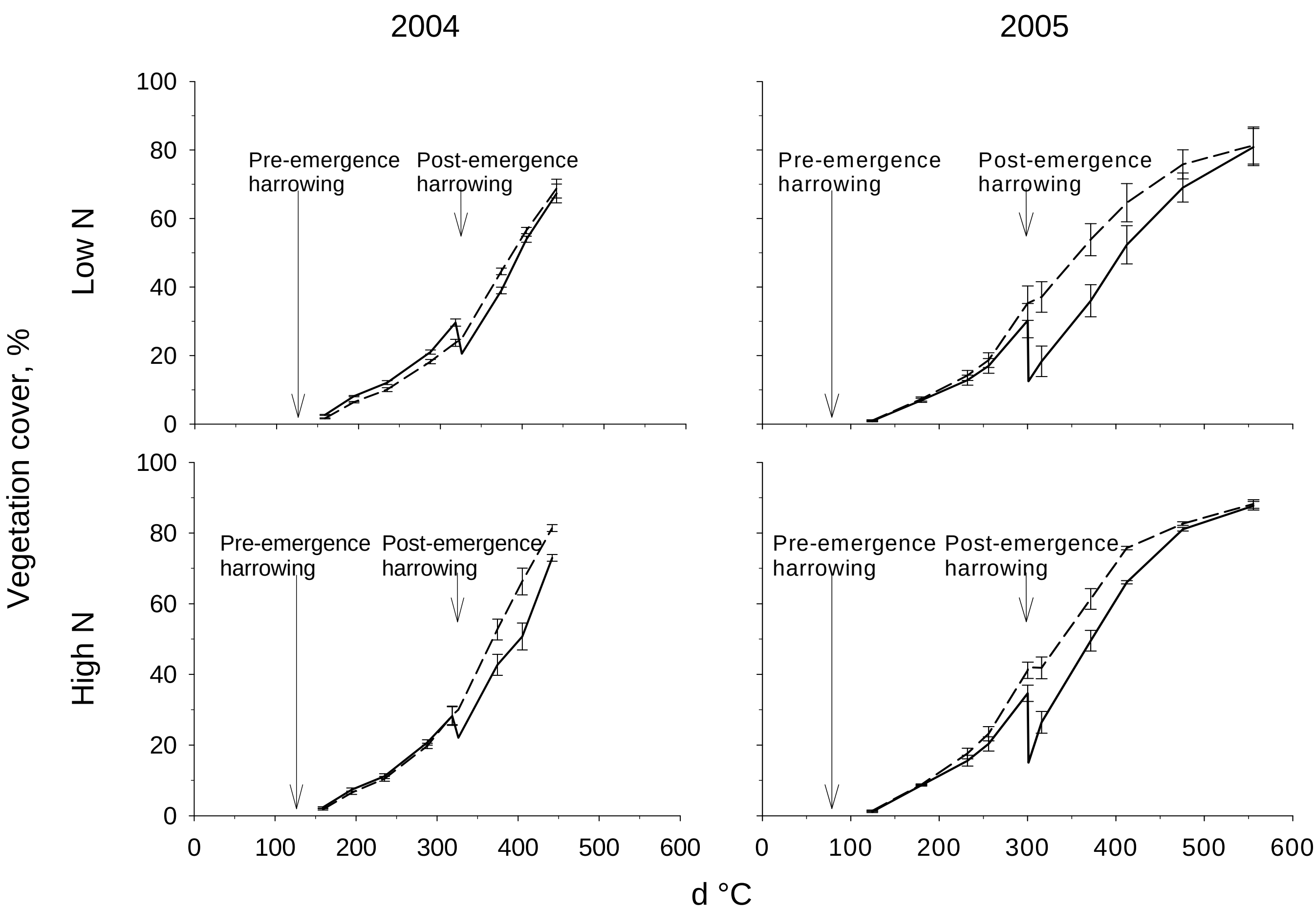


**Fig. 1** Development of vegetation cover (VC) exemplified by cv. Brazil under the low nutrient level (upper) and the high nutrient level (lower) in herbicide treated plots in 2004 (left) and 2005 (right). The solid lines show weed harrowed plots and the broken lines show non-harrowed plots. Vertical lines show standard error.

Surprisingly, pre-emergence weed-harrowing increased the crop density ($D_C$) of Modena and Brazil in both years and under both nutrient levels, by 26 and 25 plants m$^{-2}$, respectively, averaged over all other factors (Fig. 2). For Otira, $D_C$ was reduced by 11 plants m$^{-2}$ on average by weed harrowing at the high nutrient level, while there was no significant difference at low nutrient levels. Pre-emergence weed-harrowing had a negative effect on $D_C$ for Orthega in 2004 (13 plants m$^{-2}$ less) while in 2005 the opposite occurred (32 plants m$^{-2}$ more).

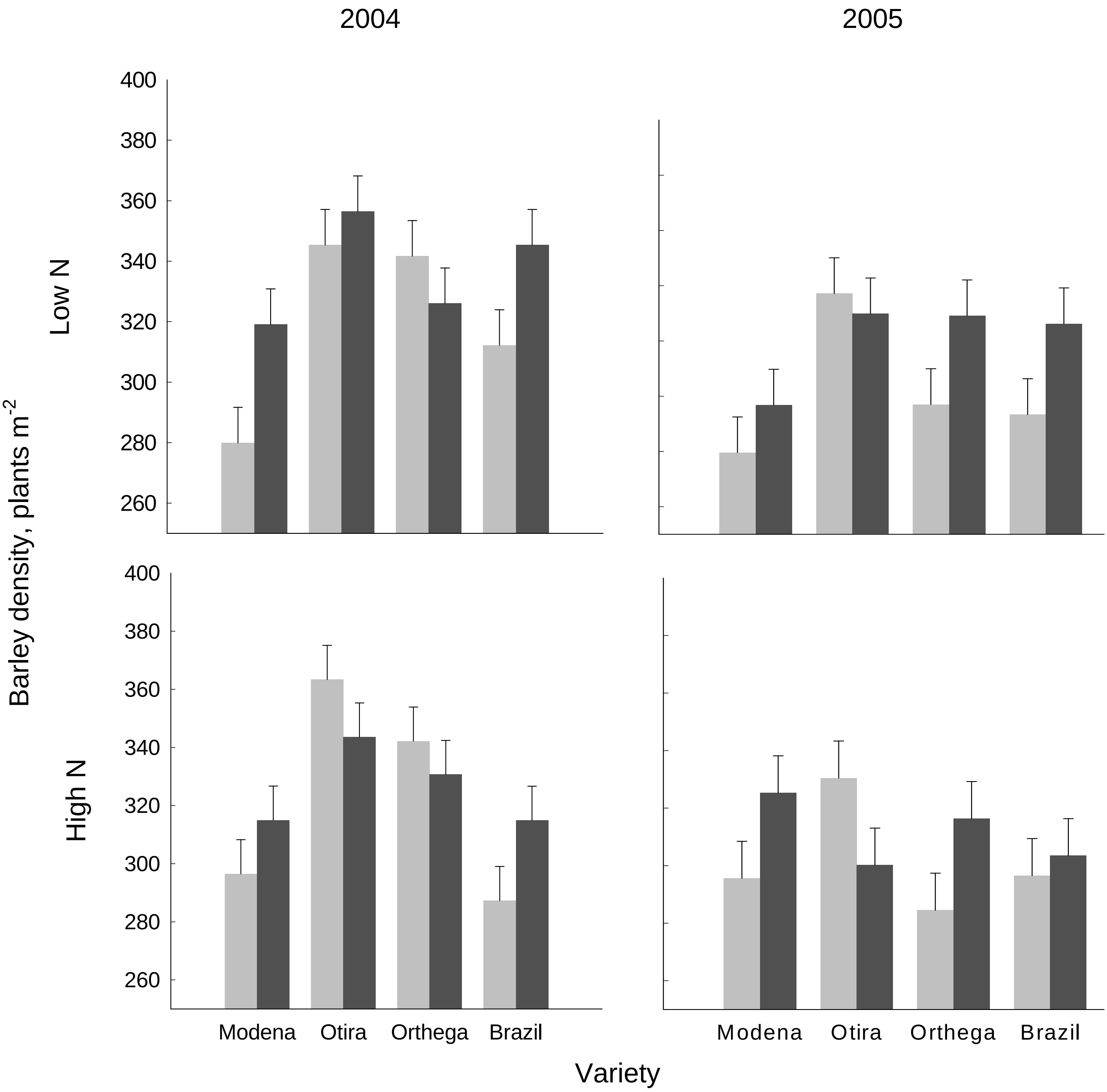


**Fig. 2** Crop plant density adjusted for experimental design, under low (upper) and high (lower) nutrient levels in 2004 (left) and 2005 (right). Light grey bars show non-harrowed plots and dark grey bars show harrowed plots. Vertical bars show standard errors.

### *Effects of post-emergence weed harrowing on vegetation cover and LAI*

The change in vegetation cover ($\Delta VC$) caused by post-emergence weed harrowing was analysed (eqn. (7)) for differences between the varieties in the herbicide-treated plots. There was a very strong effect of variety ($p<0.0001$) and year ($p<0.0001$). We found significant effects of the interaction between nutrient level and year ($p=0.039$). Orthega was covered less by harrowing than other varieties. We found only a 7 percentage point reduction in this variety compared to Otira, where the reduction was more than the double (15 percentage point). In herbicide-untreated plots, $\Delta VC$ represents the reduction in the sum of vegetation cover of both weeds and crop. There were only slight differences in the levels of $\Delta VC$, whether weeds were present or not, indicating that the main differences in $\Delta VC$ were caused by differences in crop cover (Table 3). We found a significant negative correlation between $\Delta VC$ and canopy height measured six days after weed harrowing in 2004 (low nutrient level, p<0.001; high nutrient level, p=0.007). In 2005 there was no correlation (Fig. 3).

**Table 3** Change in vegetation cover (percentage points) for herbicide-treated $(\widehat{\Delta VC_{vh+}})$ and untreated plots $(\widehat{\Delta VC_{vh-}})$. Average over two years (standard error)

| Variety | $\widehat{\Delta VC_{vh+}}$ | $\widehat{\Delta VC_{vh-}}$ |
|---|---|---|
| Modena | 0.10 (0.008) | 0.12 (0.012) |
| Otira | 0.15 (0.008) | 0.12 (0.012) |
| Orthega | 0.07 (0.008) | 0.06 (0.011) |
| Brazil | 0.13 (0.009) | 0.17 (0.011) |

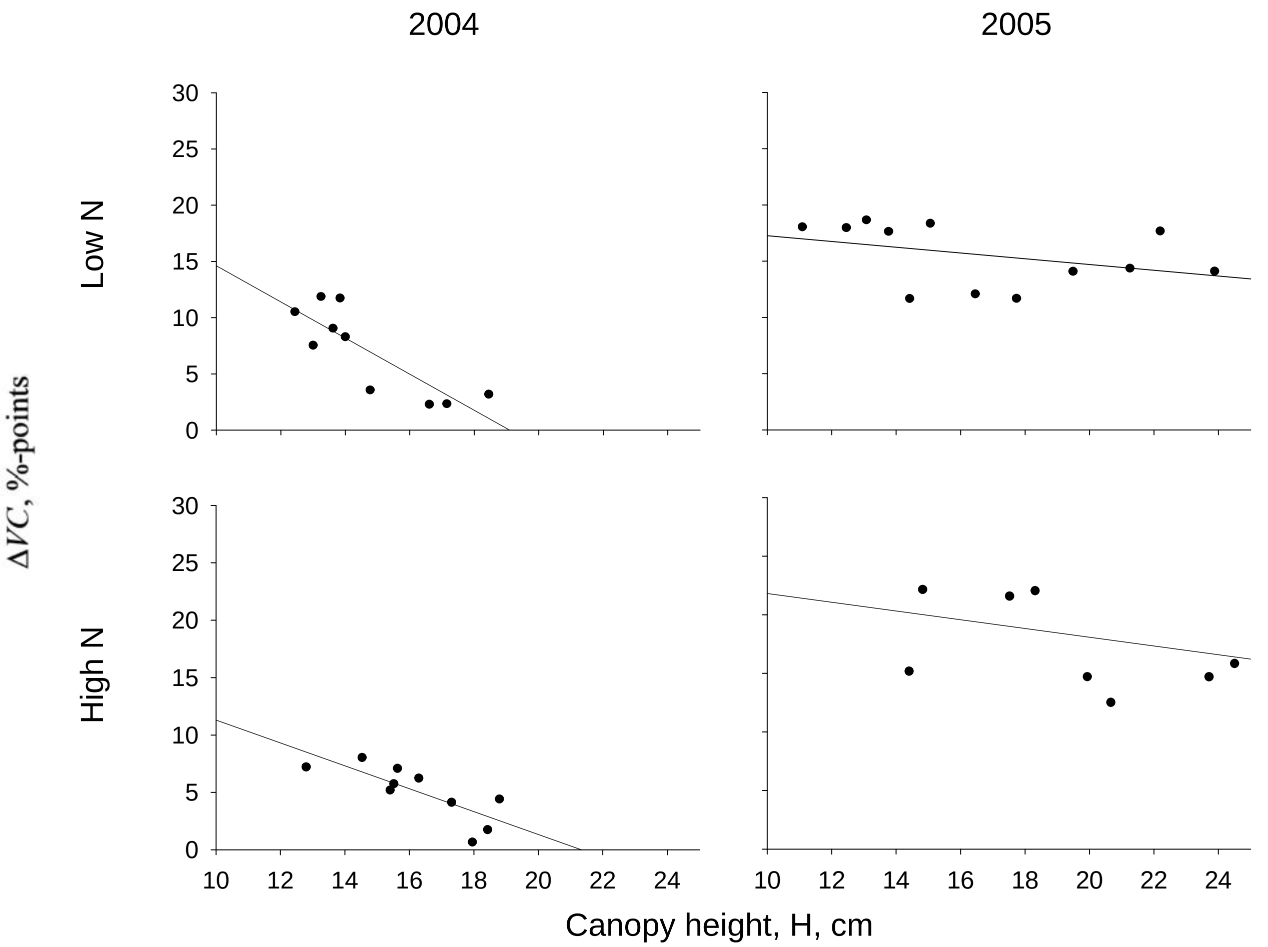


**Fig. 3** Relationship between canopy height, H and ΔVC under low (upper) and high (lower) nutrient levels in 2004 (left) and 2005 (right). Each point represents one plot. Low nutrients 2004:34.2-1.9H ($r^2$=0.69). Low nutrients 2005: 25.5-0.6H ($r^2$=0.14). High nutrients 2004: 24.0-1.2H ($r^2$=0.53). High nutrients 2005: 31.5-0.07H ($r^2$=0.26).

*LAI* measured in the herbicide-treated, non-harrowed plots approximately 2 weeks after the post-emergence weed harrowing showed a strong significant effect of variety (eqn. (7)), nutrient level and year ($p<0.0001$ for all) but with an interaction between variety and year ($p=0.006$) and between nutrient level and year (0.01) (Table 2, Fig. 4). For the change in *LAI* caused by harrowing, there was a significant difference between years, but there were no significant differences between varieties or any varietal interactions. There was no significant effect of weed harrowing on *LAI* in 2004, while in 2005 harrowing caused a 9.3% reduction in *LAI*.

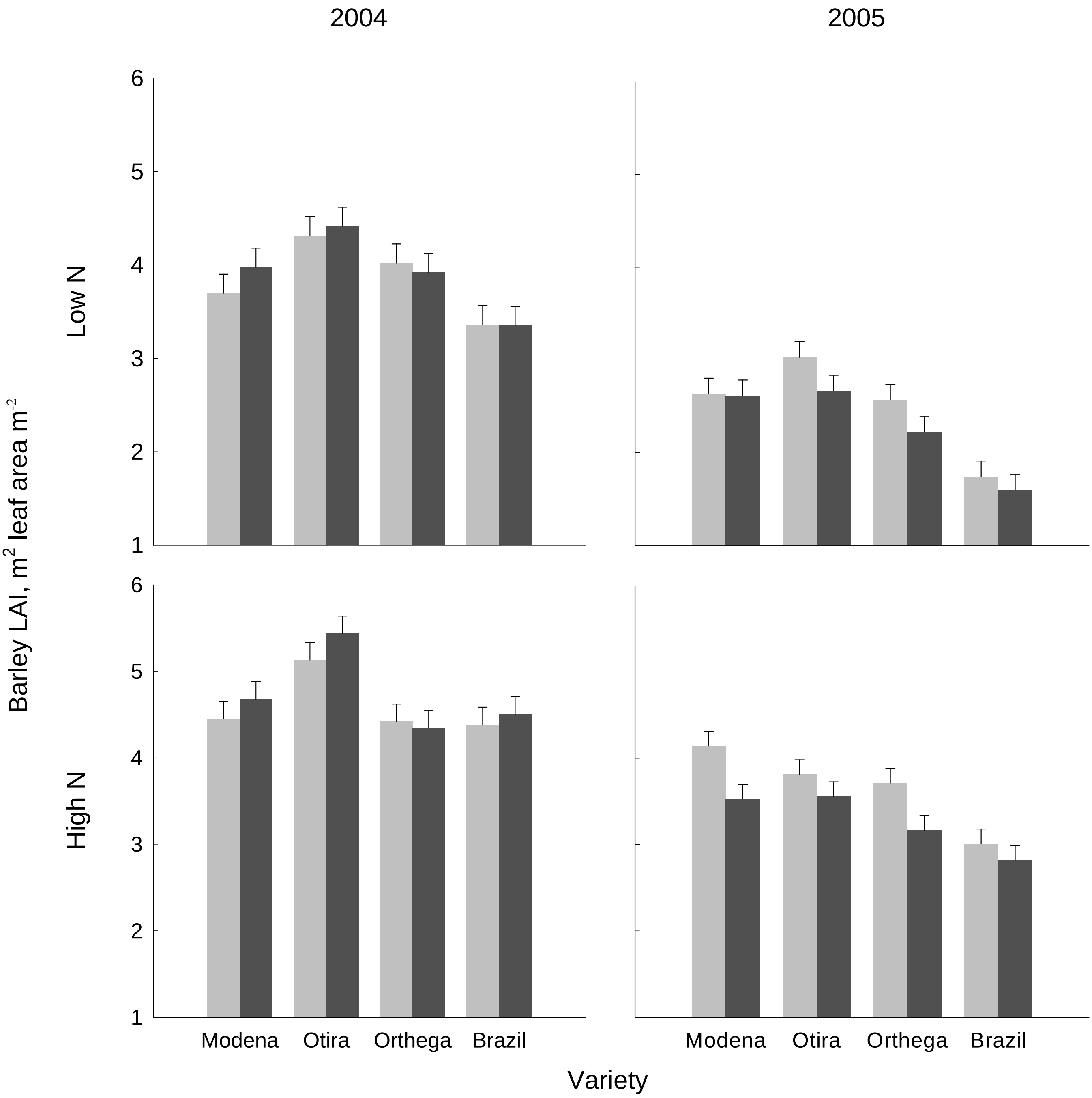


**Fig. 4** Barley LAI from herbicide treated plots adjusted for experimental design, under low (upper) and high (lower) nutrient levels in 2004 (left) and 2005 (right). Light grey bars show non-harrowed plots and dark grey bars show harrowed plots. Vertical bars indicate standard errors.

Due to the differences in crop density induced by the varietal differences in response to pre-emergence harrowing, $\Delta D$ was used as a covariate in the analysis, which gave a significant improvement of the model (eqn. (8)). The slope of $\Delta D$ was 0.000263, which means that an increase caused by the pre-emergence weed harrowing of 1 plant $m^{-2}$ would result in an increased vegetation cover of 0.026 percent.

### *Effects on weed biomass*

In the non-harrowed, herbicide-untreated plots there was significantly more weed biomass in 2005 than in 2004 (Fig. 5). The biomass production differed among the varieties (p=0.012): 114 g $m^{-2}$ for Orthega, 85.7 g $m^{-2}$ for Brazil, 74.9 g $m^{-2}$ for Modena and 67.3 g $m^{-2}$ for Otira (back-transformed averages over both years). Thus, there was 69% more weed biomass in non-harrowed and herbicide-untreated plots with Orthega compared to Otira.The only significant interaction was between nutrient level and year (p=0.032).

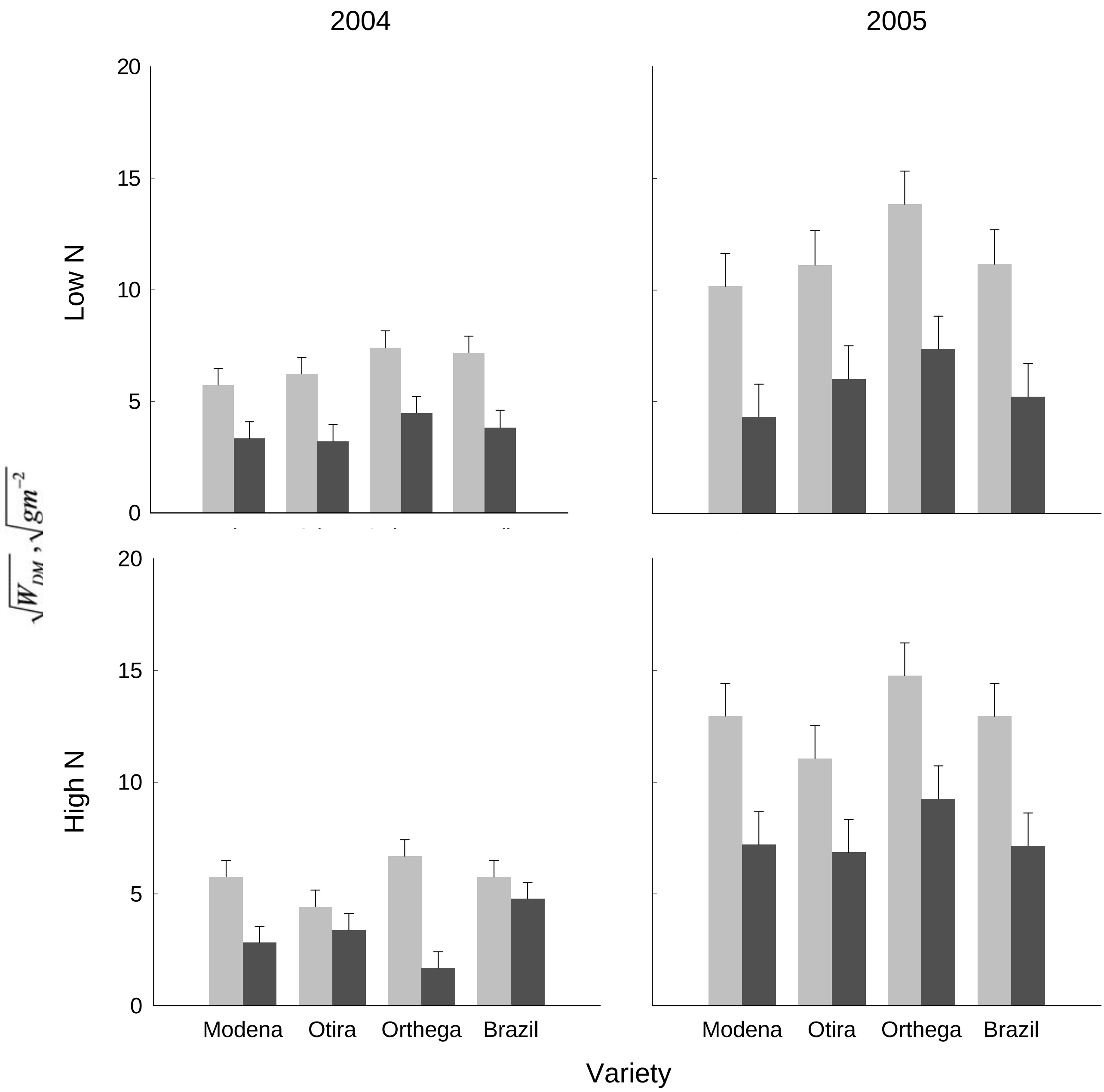


**Fig. 5** Square root transformed weed biomass adjusted for experimental design in herbicide untreated plots under low (upper) and high (lower) nutrient levels in 2004 (left) and 2005 (right). Light grey bars show non-harrowed plots and dark grey bars show harrowed plots. Vertical bars show standard errors.

In the weed-harrowed, herbicide-untreated plots, weed biomass was four times greater in 2005 than in 2004. As for the non-harrowed plots, we found a significant interaction between nutrient level and year; weed biomass was six times greater at the high nutrient level in 2005 (58 g $m^{-2}$) compared to 2004 (10 g $m^{-2}$), while at the low nutrient level there was only a three times increase from 2004 (13.8 g $m^{-2}$) to 2005 (32.8 g $m^{-2}$). We found a significant variety by year interaction (Fig. 5), as the plots seeded to Orthega had a greater amount of weed biomass (relative to plots seeded to other varieties) in 2005 versus 2004, while the opposite was true for Brazil. There were no significant interactions between variety and nutrient level. We tested if $\Delta VC$ or $\Delta D$ could explain some of the variation in weed suppression and found that there was no significant improvement of the statistical model (eqn. (8)) by adding either $\Delta VC$ or $\Delta D$ or both to the model.

An analysis of $\Delta W$ (from eqn. (7)) as well as the relative reduction in weed biomass showed no significant varietal differences, meaning that weed harrowing did not significantly affect the weed varietal suppressive ability i.e. strong weed suppressors remained strong after weed harrowing.

### *Effects on crop yield*

In the herbicide-treated plots without weed-harrowing (Fig. 6), there were a significant effects of variety ($p<0.0001$), nutrient level ($p<0.0001$) and year ($p=0.0026$), but there were significant interactions between nutrient level and year ($p=0.022$), and between variety and year ($p=0.002$). In the herbicide treated plots with weed harrowing (Fig. 6), we found a significant effect of variety ($p<0.0001$), year ($p<0.0001$) and nutrient level ($p=0.007$), but yields responded differently at different nutrient levels in the two years ($p=0.0004$). In 2004 there was no significant difference between the nutrient levels. In 2005 there was a strong significant difference with 7.1 hkg $ha^{-1}$ increase from the low to the high nutrient level (Fig. 6). We found a tendency for interaction between variety and nutrient level in the herbicide treated plots that were harrowed ($p=0.068$). This was caused by the relatively much greater yield response of Modena to the high nutrient level treatment in comparison to the other varieties.

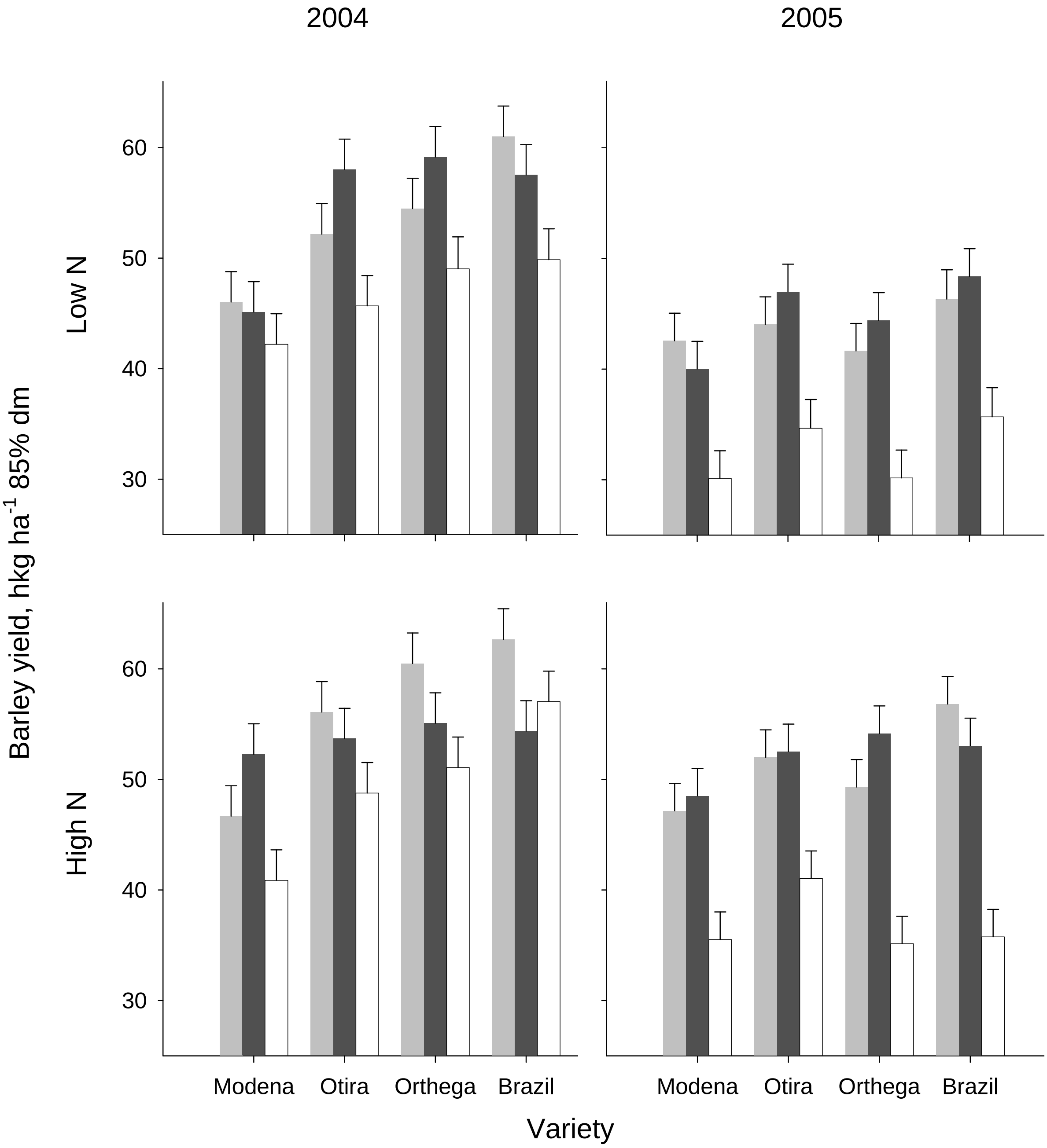


**Fig. 6** Yield of the varieties in 2004 and 2005 under low (upper) and high (lower) nutrient levels in 2004 (left) and 2005 (right) in herbicide treated plots. Light grey bars show non-harrowed plots and dark grey bars show harrowed plots. White bars show barley yields from weedy plots. Vertical bars show standard errors.

The estimated yield difference between harrowed and not harrowed treatments (eqn. (5)) (light grey bar minus dark grey bars in Fig. 6) showed a strong tendency for varietal differences ($p=0.057$), but this varietal effect interacted significantly with the nutrient level ($p=0.041$). As a mean of the two years, Otira benefited significantly from the harrowing treatment (a yield increase of 4.4 hkg $ha^{-1}$ at the low nutrient level). In contrast, for Brazil we found a marked yield reduction as a result of harrowing in the high nutrient level treatments (a yield decrease of 6.0 hkg $ha^{-1}$). Due to the marked varietal differences in crop density, we tested $\Delta D$ as a covariate, but it did not significantly improve the model.

## Discussion

We found a significant negative effect of pre-emergence weed harrowing on vegetation cover (*VC)* at the high nutrient level, but no significant differences between harrowed and non-harrowed at the low nutrient level (Fig. 1). This interaction indicates that the negative effects on *VC*, which usually are observed after pre-emergence weed harrowing (J. Rasmussen, pers. comm.), were reduced by a compensatory positive effect at low nutrient levels in varieties like Modena and Brazil. This could be the result of breaking a crusty soil surface or increasing soil temperature, oxygen levels and nitrogen mineralization. In a study with two to three post-emergence weed harrowings in spring wheat under conditions without any applied fertiliser, Steinmann (2002) concluded, that post-emergence harrowing had only minor effect on the nutritional status in the crop, but that the nitrogen content in the soil was increased significantly. We applied 40% of the optimal crop requirement (Anon, 2003) at the low nutrient level. At this level a minor increase in nitrogen mineralization could compensate for damage caused by pre-emergence harrowing and weed harrowing could be a more suitable and viable practice under conditions of low versus high nutrient levels.

For Modena and Brazil the positive effect of pre-emergence weed harrowing was expressed as a marked increase in crop density (Fig. 2). The varietal differences in crop density response could be caused by differences in speed of germination or vigour (Rasmussen & Rasmussen, 1999), emergence force (Bouaziz *et al.,* 1990), or in response to changes in nutrient level, aeration and temperature caused by harrowing (Steinmann, 2002). Bouaziz *et al.* (1990) found that a winter wheat variety had 100% emergence when obstacles (clods etc.) below 25g were removed, and the emergence reduced linearly with increasing obstacle size. The study of Bouaziz *et al.* (1990) included only one variety and there could have been an effect due to varietal differences in emergence force.

The marked varietal differences in resistance, measured as change in vegetation cover ($\Delta VC$, Table 3), could be explained by the differences in plant height at the time of harrowing (Table 2, Fig. 3). Similarly Kurstjens and Perdok (2000) found a linear correlation between percentage coverage and plant height for ryegrass (*Lolium perenne* L.). Thus varieties, which are high at the time of harrowing, are more likely to resist damage caused by post-emergence harrowing, and such varieties should be chosen if weed harrowing is planned.

There was a small but significant positive correlation between the change in crop density $\Delta D$ and $\Delta VC$. The increase of 25 plants $m^{-2}$ due to pre-emergence weed-harrowing, found for Modena and Brazil, corresponded to an increase in coverage caused by post-emergence weed-harrowing of 25 × 0.026 = 0.65 percentage points. This could be due to smaller and less cover-resistant crop plants in the varieties that showed an increase in crop density due to the pre-emergence harrowing treatment. Thus varieties, which are tall at post-emergence harrowing and have increased density after pre-emergence harrowing, are the ones that benefit most from weed harrowing.

We did not find any significant reduction in *LAI* caused by harrowing in 2004, while there was a significant 9.3% reduction in *LAI* on average in 2005 (Fig. 4). Similarly Rasmussen *et al.* (2004) found negative but non-significant effects on *LAI*. The efficacy of weed-harrowing is very dependent on weather and soil conditions, and differences among years are therefore also expected. We found no significant varietal interactions with weed-harrowing, indicating that weed-harrowing affects *LAI* in an additive fashion.

Rasmussen *et al.* (2004) found that varieties responded differently to weed harrowing when measured on relative yield reduction and that the yield response was negatively correlated with parameters associated with competitive ability. Rasmussen *et al.* (2004) showed that the yield of high yielding varieties was affected significantly more than that of low yielding varieties, however, there was still an overall yield benefit from choosing high versus low yielding varieties, even when plots were harrowed. In the study of Rasmussen *et al.* (2004), there was an interaction with disease severity, as mildew tended to be more aggressive in short (less suppressive) varieties. We used the absolute yield difference between harrowed and not-harrowed plots thus eliminating the possible effects of different levels of diseases. We found very different varietal responses on crop yield, as weed harrowing was significantly beneficial for Otira, while Brazil suffered from weed harrowing. Brazil tended to be the highest yielding variety under herbicide-treated, non-harrowed conditions at both high and low nutrient levels, while Otira had an intermediate yield. We found that the highest yielding varieties did not always result in the highest yield because of differences amongst varieties in their tolerance to weed-harrowing.

We found that a variety with strong weed suppressive ability remains a strong weed suppressive variety whether or not weed-harrowing is used. Rasmussen and Svenningsen (1995) studied the interaction between row distance and three spring barley varieties in an experiment with no pre-emergence weed harrowings and two post-emergence harrowings with one-month time interval. With respect to weed control efficacy, they did not find any significant interactions between variety and harrowing treatment.

Under organic or low-input growing conditions with high weed pressure, Otira would be a good choice of variety due to its strong suppressive ability in combination with a positive response to weed harrowing and relatively high yield. In contrast Brazil despite high yields, suffered from weed harrowing and had less weed suppressive ability. In conclusion, this study shows that the varieties differed in their response to weed harrowing, in terms of yield but not in terms of weed suppressive ability. Moreover the yield response interacted with the nutrient level.

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# Paper III. Can early sensor measurements be used to predict the yield of spring barley (*Hordeum vulgare* L.) varieties in competition with weeds?

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**Short title:** Can early sensor measurements predict spring barley yield?

## Summary

At the time of weed control, crop yield and potential yield loss caused by weeds are both uncertain because only a minor part of the growing season has passed and the period of yield formation lies after weed control.

The aim of this study was to investigate the feasibility of using sensor-based measurements of reflectance, vegetation cover and canopy structure as well as weed density in the early growth stages of the crop to estimate spring barley yield under different conditions. For two years four spring barley varieties were grown either in pure stands or in mixtures with and without competition from weeds at two different nutrient levels.

The results showed that by using 14 reflectance measurements conducted through the entire growing season as well as measurement of weed density and two canopy structure measurements, a multivariate ordination technique using partial least squares (PLS) was able to explain 65% of the yield variation with seven principal components (PCs). By excluding weed density and the canopy structure measurements the predictability of the PLS model was not reduced. By using only the first five sensor-based measurements (before crop growth stage 21-22), the PLS model could explain 38% of the yield variation. Further reductions in

the numbers of measurements reduced the accuracy of the model; however, we found that a measurement 16-18 days after sowing alone explained 27% of the variation in yield.

Compared to the variation explained by using all available measurements through the entire growing season, an early sensor-based measurement can give reasonable estimates of the expected yield helping the farmer to optimise the use of herbicides.

**Keywords**: Multivariate regression; reflectance; image analysis; competition; yield; weeds

## Introduction

In spring barley, the major part of the weed control is conducted before growth stage 21-23 BBCH (Lancashire *et al.,* 1991). At that time it is difficult to predict the eventual crop yield. Knowledge of potential crop yield is necessary to assess the yield loss potentially caused by weeds. The main difficulty of predicting crop yield early on in the season reason is that the weather in the remaining, major part of the growing season is decisive for yield formation. Nevertheless, this is the ideal time for optimising weed control, while weeds are small and crop-weed competition has hardly begun.

Several decision support systems use weed density and growth stage at the time of weed control as input for decision algorithms, e.g. Crop Protection Online (Anonymous, 2005a) or WeedSoft (Hock *et al.,* 2006)). Weed density and crop yield loss have been shown to follow a robust hyperbolic relation (Cousens, 1985) with asymptote and slope dependent on weed species, its growth stage and the crop (Holst, 2005). But weed density is laborious to assess, especially when one is aiming at site-specific weed management. Therefore it would be useful if it was possible to achieve a reliable crop yield estimate before weed control by automatic non-destructive sensor-based measurements in the early season.

In weed-free crops it is possible to use non-destructive measurements in late growth stages for predicting the yield with reasonable accuracy (Hansen *et al.,* 2003). Under weedy conditions several researchers have attempted to predict yield loss due to weeds by measuring the relative weed/total leaf area in the early growth stages (Lotz *et al.,* 1992; Kropff *et al.,* 1995; Lotz *et al.,* 1996; Ngouajio *et al.,* 1999c). This method gives a better description of the yield loss due to weeds compared to the density model, especially when the weeds emerge in flushes. However, there are some complications in using the method: 1) there is a need for *automatic* data acquisition to distinguish leaf area of crop and weeds, which is not possible in cereals yet, 2) the leaf area of the weeds must be combined with information of which species are present and the relative distribution of the species, as the competitive ability of weed species differs, 3) the method has till now shown too inaccurate yield loss predictions in sugar beet (*Beta vulgaris,* L.) and spring wheat (*Triticum aestivum,* L.) in competition with *Sinapis alba,* L to be been used in decision-making systems for integrated weed management (Lotz *et al.,* 1996).

Some researchers have used image analysis to measure relative leaf cover (the vertical projection of plant canopy on the ground) in maize (Ngouajio *et al.,* 1999a; Ngouajio *et al.,* 1999b), sugar beets (Heisel *et al.,* 2002) and vegetables (Grundy *et al.,* 2005). Other methods to discriminate crop and weed include chlorophyll fluorescence profiles (Keränen *et al.,* 2003), indices of plant reflectance spectra (Wiegand *et al.,* 1990; Vrindts *et al.,* 2002) and advanced image analysis methods (Andreasen *et al.,* 1997; Søgaard, 2005), as reviewed by Gerhards and Christensen (2003). All these methods use optoelectronic sensors or CCD cameras to measure reflectance in the green, red and often also near-infrared (NIR) wave lengths. Green leaves are characterised by a high reflectance in green and near-infrared wavelengths and low reflectance in the red spectrum compared with the reflectance from bare soil.

By combining output from different types of sensors, i.e. sensor fusion, measurement quality can be improved for instance for fruit quality assessments (Steinmetz *et al.,* 1999) or for monitoring sprayer boom movements (Ooms *et al.,* 2002) The statistical method used for prediction analysis must be able to handle multivariate data structures with high covariance and redundancy. This requirement is fulfilled by partial least squares regression (PLS) models (Rännar *et al.,* 1995; Kenkel *et al.,* 2002).

The aim of this study was to use a variety of sensor-based non-destructive measurements in the early season, before weed control, to measure the reflectance of the cropped area with or without weeds. The accuracy of yield prediction from these measurements was compared with the accuracy achieved by estimates based on non-destructive measurements from the entire growing season. Although the accuracy increased markedly by including later measurements in the predictive model, much predictive power remained even when only a few early measurements were included.

## Materials and methods

### *Experimental conditions*

Four varieties of spring barley (1=Modena; 2=Otira; 3=Orthega; and 4=Brazil) were chosen to represent the range in varietal weed suppressiveness among varieties on the Danish variety list (Anonymous, 2005b; Hansen *et al.,* 2007a). The varieties as well as three two-component mixtures (5=50% Modena + 50% Otira; 6=50% Modena + 50% Orthega; 7=50% Modena + 50% Brazil) and one three-component mixture (8=33% Modena + 33% Otira + 33% Orthega) of the varieties were studied in field trials at Flakkebjerg, Denmark (55°19'N, 11°24'E) in 2004 and 2005. The field trials were split-plot designs. Whole plots consisted of the eight combinations of three factors; two levels each of herbicides (±), weed harrowing (±), and nutrient level (40% or 80% of the recommended nitrogen need) (Anonymous, 2003). However, only plots without weed harrowing are considered in this paper. The crop was sown with a seed drill with 12.0 cm row spacing on 15 April 2004 and 13 April 2005. Seed rate was adjusted for seed weights and germination rate to give a target established population of 350 plants $m^{-2}$. As model weeds we used a mixture of 25% viable seeds of *Chenopodium album* L., 25% *Phaselia tanacetifolia* Benth*.,* 25% *Brassica napus ssp. napus* L. and 25% *Trifolium incarnatum* L. *cv.* Poppelsdorfer in plots with no herbicide treatments. The weeds were sown 16 April 2004 and 13 April 2005 at a density of 200 seeds $m^{-2}$. Naturally occurring weeds were *Stellaria media* (L.) Vill., *Sinapsis arvensis* L., *Viola arvensis* Murray*, Veronica arvensis* L.*, Thlaspi arvense* L. and *Polygonum convolvulus* L.. The total density of these species did not exceed 50 plants $m^{-2}$. Further details of the experiment can be found in Hansen *et al.* (2007b).

### *Measurements*

Throughout the growing season several non-destructive measurements were conducted (Table 1). Canopy reflectance was measured with a hand-held spectroradiometer fitted with 20.8° field-of-view optics (CropScan MSR16, CropScan Inc., USA). Twelve medium broadbands (≅ 10 nm) were used, with centre wavelengths equal to 460, 510, 530, 560, 610, 660, 710, 730, 760, 780, 810 and 950 nm. Both solar irradiation and ground/crop reflectance were detected. All measurements were conducted between 10:00 and 14:00. The data used in the analysis were the relative reflectance corrected for irradiation referred to as reflectance data.

The reflectance data were further used for calculating seven different vegetation indices (Table 2).

**Table 1** Dates of sensor measurement and crop yield measurement. Data marked with "x" indicate which data were used in the four scenarios

| | Date | | Scenario | | | | |
|---|---|---|---|---|---|---|---|
| Task and measurement no. | 2004 | 2005 | 1.1 | 1.2 | 2.1 | 2.2 | 3.1-3.12** |
| Image acquisition 1 | 30-Apr | 28 Apr | x | x | x | x | x |
| Reflectance measurements 1 | 30-Apr | 27 Apr | x | x | x | x | x |
| Image acquisition 2 | 03 May | 03 May | x | x | x | x | x |
| Reflectance measurements 2 | 03 May | 03 May | x | x | x | x | x |
| Density, weeds | 04 May | 19 May | x | | x | | |
| Image acquisition 3 | 06 May | 09 May | x | x | x | x | x |
| Reflectance measurements 3 | 06 May | 09 May | x | x | x | x | x |
| Image acquisition 4 | 10 May | 12 May | x | x | x | x | x |
| Reflectance measurements 4 | 10 May | 12 May | x | x | x | x | x |
| Image acquisition 5* | 13 May | 17 May | x | x | x | x | x |
| Reflectance measurements 5* | 13 May | 17 May | x | x | x | x | x |
| Image acquisition 6 | 14 May | 18 May | x | x | | | |
| Reflectance measurements 6 | 14 May | 18 May | x | x | | | |
| Image acquisition 7 | 18 May | 19 May | x | x | | | |
| Reflectance measurements 7 | 18 May | 20 May | x | x | | | |
| Image acquisition 8 | 21 May | 23 May | x | x | | | |
| Reflectance measurements 8 | 21 May | 23 May | x | x | | | |
| Image acquisition 9 | 25 May | 26 May | x | x | | | |
| Reflectance measurements 9 | 25 May | 26 May | x | x | | | |
| Reflectance measurements 10 | 02 June | 31 May | x | x | | | |
| Image acquisition 10 | 07 June | 06 June | x | x | | | |
| Canopy measurements 1 | 07 June | 01 June | x | | | | |
| Reflectance measurements 11 | 14 June | 06 June | x | x | | | |
| Reflectance measurements 12 | 21 June | 20 June | x | x | | | |
| Canopy measurements 2 | 23 June | 17 June | x | | | | |
| Reflectance measurements 13 | 07 July | 04 July | x | x | | | |
| Reflectance measurements 14 | 20 July | 27 July | x | x | | | |
| Crop yield | 04-aug | 16-aug | x | x | x | x | x |

*Before GS 21-22
** measurements from max two dates are analysed in this scenario

**Table 2** Vegetation indices calculated from CropScan measurements

| Name | Equation | Reference |
|---|---|---|
| RVI | $\frac{R810}{R660}$ | (Christensen & Goudriaan, 1993) |
| NDVI | $\frac{(R810-R690)}{(R810+R690)}$ | (Rouse *et al.,* 1974) |
| TVI | $0.5\times(130\times(R810-R560)-210\times(R690-R560))$ | (Broge & Leblanc, 2001) |
| REIP | $700+40\times\frac{\frac{R660+R780}{2}-R710}{R730-R710}$ | (Dawson & Curran, 1998) |
| D-chl-ab | $\frac{(R760-R740)/2}{R560}$ | (Gitelson & Merzlyak, 1996) |
| $\mathrm{VARI_g}$ | $\frac{R560-R660}{R560+R660-R460}$ | (Gitelson *et al.,* 2002) |
| RGI | $\frac{R560-R660}{R560+R660}$ | (Tillet *et al.,* 2001) |

Within 15h of the time of reflectance measurement, soil coverage was measured by taking a digital image (Canon PowerShot G1 Camera) at two positions inside each plot. The exact positions were marked to ensure that reflectance measurements and the images were acquired at the same spot every time. A detailed description of the image processing procedure and analysis can be found in Hansen *et al.* (2007b).

Leaf area index (*LAI*; $m^2$ leaf area per $m^2$ ground area), diffuse non-intercepted radiation (DIFN) and leaf angle (MTA; °, where 90° indicates vertical leaves and 0° indicates horizontal leaves) were measured at two dates in 2004 and 2005 (Table 1). We measured twice per plot at the same positions as the other measurements using LICOR 2000 Canopy Analyzer (Lang *et al.,* 1985; Welles & Norman, 1991).

Weed density was recorded on 4 May 2004 and 1 May 2005 as the number of *Chenopodium album* L., (*HVI*), *Phaselia tanacetifolia* Benth.,(*HON*) *Brassica napus ssp. napus* L., (*RAP*), *Trifolium incarnatum* L. *cv.* Poppelsdorfer (*BLO*), perennial weeds (*ROD*) and other weeds (*AND*) in plots with no pesticide treatments. The densities were recorded twice in every plot in a 0.5×0.5 m frame and recalculated to plants $m^{-2}$ at the same positions as the other measurements.

Crop yield, ($Y$, hkg $ha^{-1}$) was measured 4 August 2004 and 16 August 2005 in a 0.5×0.5 m frame at the same positions as the other measurements by cutting the above-ground crop material and threshing the material with an experimental thresher. The grain samples were dried at 100°C for 24 hours and dry matter was measured. Afterwards the yield was adjusted to 85% dry matter.

Altogether, the analysed data consisted of 288 variables and 384 samples.

### *Statistics*

Due to the multivariate data structure characterised by a high covariance and redundancy, the analysis was conducted by the Partial Least Square regression procedure (PROC PLS) in SAS (SAS Institute Inc., 1999). The PLS algorithm choose successive orthogonal factors that maximize the covariance between each X score and the corresponding Y score. We used the RLGW algorithm, which is an iterative approach that is efficient when there are many predictors and few response variables (Ränner *et al.,* 1994; Rännar *et al.,* 1995).

The initial PLS was conducted on raw data with centering $(x-\bar{x})$ and scaling using 1/St.dev with one analysis common for the two years. Due to a clear grouping of the scores between years (data not shown), data were standardised within year before entering the final PLS procedures, hereby eliminating the strong effect of year and herbicide treatment. The standardisation was performed in PROC STANDARD (SAS Institute Inc., 1999) with mean=0 and standard deviation=1, and missing values replaced with 0. No outliers were detected.

The approximate optimal predicted residuals (PRESS) were determined by "leave-one-out cross validation" (block size = 2, which equals one cross validation block per plot) and the optimal numbers of principal components (PC) were found where root mean PRESS was at its minimum. Cross validation means that two responses were excluded from the dataset in turn and the responses left out were predicted using the model calculated from the remaining part of the responses (Rännar *et al.,* 1995). The predictors were examined for their relative importance by plotting their weights based on the selected PCs against each other. Predictors, close to the origin of the weight plot, do not contribute much to the prediction, and the regression coefficients (B) of these predictors represent the importance of each predictor in predicting the response. Furthermore, the Variable Importance in the Projection (VIP) (Chong & Jun, 2005) was calculated by

$$VIP = \sqrt{\frac{p\sum_{k=1}^{h}\left(ss\left(b_k\boldsymbol{t}_k\right)\left(\frac{w_{jk}}{\|w_k\|^2}\right)\right)}{\sum_{k=1}^{h}\left(ss\left(b_k\boldsymbol{t}_k\right)\right)}} \tag{1}$$

where $\boldsymbol{t}_k$ is the $k^{\text{th}}$ score vector and $w_{\text{jk}}$ is its associated weight vector for the column vector $k$ and the variable $j$. The VIP represents the value of each predictor in fitting the PLS model for both predictors and response. If a VIP of a predictor was less that 1.0 (Chong & Jun, 2005), and the predictors had a small absolute B, we eliminated these predictors from further analysis.

To test for systematic divergence of the PLS model, the following MIXED model (SAS Institute Inc., 1999) was conducted on the residuals of the predictions

$$\begin{aligned}\widehat{Y_{nvgy}} - Y_{nvgy} = \mu_2 + \alpha_{2,n} + \delta_{2,v} + \lambda_{2,y} + &[\text{all two-factor interactions}] + \\ &[\text{the three-factor interactions}] + \\ &E_{21,g} + E_{22,gy} + E_{23,gny} + E_{24,gnyv}\end{aligned} \tag{2}$$

where $\widehat{Y_{nvgy}}$ indicates the predicted yield by the PLS models for variety $v$ (regarding each variety mixture as a 'variety') treated with nutrient level $n$, of replicate $g$ in year, $y$. $E_{21,g}$ is the random effect of replicate $g$. $E_{22,gy}$ is the random effect of the interaction between replicate and year and $E_{23,gny}$ is the random effect of the interaction between replicate, nutrient level and year. $E_{24,gnyv}$ is the residual effect. All random effects were assumed to be normally distributed with zero mean and constant variances. Greek letters indicate systematic effects. The statistical analyses were done by the restricted maximum likelihood method in the mixed linear model procedure (SAS Institute Inc., 1999).

The PLS analyses were conducted for three scenarios for weedy and weed-free situations (Table 3). The measurements included in each scenario are shown in Table 1.

**Table 3** Tested scenarios for both weed-free and weedy plots

| Scenario | Description |
|---|---|
| how accurately can yield be predicted? | |
| *1* | *Using data from the entire growing season* |
| 1.1 | when all available non-destructive measurements from sowing until harvest are used (all data from the reflectance measurements, the image analyses, canopy structure measurements and weed density) |
| 1.2 | when all available sensor-based measurements from sowing until harvest are used (all data from the reflectance measurements and the image analyses), |
| *2* | when using sensor based measurements before growth stage 21-22 (time for the post emergence weed control) |
| 2.1 | when data from the first 5 measurements from the reflectance measurements and image analyses excl weed density recordings are used |
| 2.2 | when sensor based measurements before growth stage 21-22 are used (data from the first 5 measurements from the reflectance measurements and image analyses excl weed density recordings) |
| *3* | If only two times for measurement is possible before GS 21-22, which time is the best? |
| *4* | If only one time for measurement is possible before GS 21-22, which time is the best? |

## Results

The initial analysis showed very strong yield differences between 2004 (mean yield was 66.6 hkg ha$^{-1}$, and the standard deviation was 11.7 hkg ha$^{-1}$) and 2005 (mean yield was 45.1 hkg ha$^{-1}$, and the standard deviation was 10.2 hkg ha$^{-1}$). By standardisation within years this effect was eliminated from the further analyses.

The PLS analysis with data from reflectance, canopy structure and image analysis measurement as well as weed density recordings (standardised within years) showed that the PLS model in scenario 1.1 was able to explain 65% of the yield variation in the experiments (Table 4) by using seven PCs. The first and the second PC were closely correlated with herbicide treatment and nutrient level, respectively, while there was no clear pattern of the distribution of the varieties between the PCs (Fig. 1).

The predicted standardised values from the model are plotted against the observed standardised yields in Fig. 2. This figure shows that the model is capable of predicting yield under these large experimentally designed variations without any systematic divergence. This was also checked by eqn. (2) testing whether the residuals from the predictions of the PLS model were significantly different from zero according to genotype, nutrient level, year and the interactions. We found no significant main effects or interactions. However, by comparing the estimated least significant means from individual varieties, we found that the estimated residuals in weed-free plots with Orthega were significantly different from 0 ($p=0.024$; Table 5) and thus the model underestimated the yield of this variety. A tendency of overestimation of the yield in the Modena+Orthega mixture in the weedy plots was found too ($p=0.081$).

All the scenarios were analysed by the same model and the results are shown in Table 6. The VIP analysis (Table 7) showed that the second vegetation cover analysis (COV_2) together with results from the latest reflectance measurement (measurements named with "_14") and the weed density registrations were the most important predictors for the yield estimation.

By eliminating predictors with VIP<1.00 (Table 6) the predictive ability dropped only little (64%) still with seven PCs. However, by excluding information on weed density and canopy structure (LAI, MTA and DIFN) and thereby only using reflectance and vegetation cover measurements (scenario 1.2) the degree of yield explanation decreased markedly from 64% to 52% with four PCs.

By using only the sensor-based measurements excluding weed density recordings, the model explained 80% of the X variation and the explained Y variation was increased to 54% with eight PCs. By excluding variables with VIP<1.00 the model explained 87% and 50% of the X and Y variation respectively with five PCs.

Note that by using scenario 3.5 (sensor-based data for date 2 and date 3) the model was able to explain 32% of the yield variation, which was mainly caused by herbicide and nutrient level as well as variety, as the variation between years were eliminated by standardisation (Fig. 3).

If the measurements were conducted on date 2 (18 days after sowing; scenario 4.2), the model was able to explain 27% of the variation in yield measured 3 months later. Again the variation was mainly caused by the differences in weed control (Fig. 4). Both in scenario 3.5 and in scenario 4.2 measurements on date 2 were included, indicating that measuring growth at this time is important for estimating yield. The explained yield variation in combination with root mean PRESS indicated that scenario 3.1 3.5, 3.6 or 3.7 have a relatively high degree of predictability.

We analysed whether the different scenarios were able to predict the treatment effects by a mixed linear analysis of the residuals from (Table 8). The analyses showed that reducing the number of sensor measurements introduced significant effect of genotype, which was independent of the level of weed control. Studying the individual genotypes revealed a significant overestimation for Modena, Modena+Orthega and Modena+Brazil in the weedy plots, while the yield was underestimated in weed free plots with Otira and Orthega (data not shown).

We found a strongly significant interaction between weed control level and nutrient level, as the reduced models generally gave a small overestimation of the yield in weedy plots with low nutrient level (low yield) while the weed-free plots with high nutrient level (high yield) were markedly underestimated.

**Table 4** Percent variation accounted for by increasing numbers of PCs and the corresponding cross validation for all plots.

| | Model effects | | Dependent variables | | Cross validation |
|---|---|---|---|---|---|
| PC no. | current | total | current | total | Root mean PRESS |
| 0 | | | | | 1.414 |
| 1 | 34.54 | 34.54 | 24.53 | 24.53 | 1.240 |
| 2 | 11.10 | 45.64 | 17.66 | 42.19 | 1.123 |
| 3 | 11.66 | 57.30 | 6.70 | 48.89 | 1.103 |
| 4 | 9.29 | 66.59 | 6.00 | 54.89 | 1.079 |
| 5 | 4.99 | 71.58 | 5.81 | 60.71 | 1.062 |
| 6 | 4.54 | 76.12 | 2.20 | 62.91 | 1.013 |
| 7 | 1.76 | 77.88 | 2.08 | 64.99 | 0.981 |

**Table 5** Least Squares means of residuals of from scenario 1.1. SEP=0.133 (weed-free), SEP=0.120 (weedy).

| | weed free | | weedy | |
|---|---|---|---|---|
| Variety | Estimate | P>\|t\| | Estimate | P>\|t\| |
| Modena | -0.210 | 0.122 | -0.046 | 0.705 |
| Otira | 0.131 | 0.330 | 0.035 | 0.771 |
| Orthega | 0.311 | 0.024 | -0.038 | 0.756 |
| Brazil | -0.073 | 0.586 | -0.039 | 0.746 |
| Mo+Ot | -0.145 | 0.283 | 0.098 | 0.419 |
| Mo+Or | 0.227 | 0.095 | -0.212 | 0.081 |
| Mo+Br | 0.015 | 0.912 | -0.018 | 0.880 |
| Mo+Ot+Or | -0.048 | 0.721 | 0.011 | 0.926 |

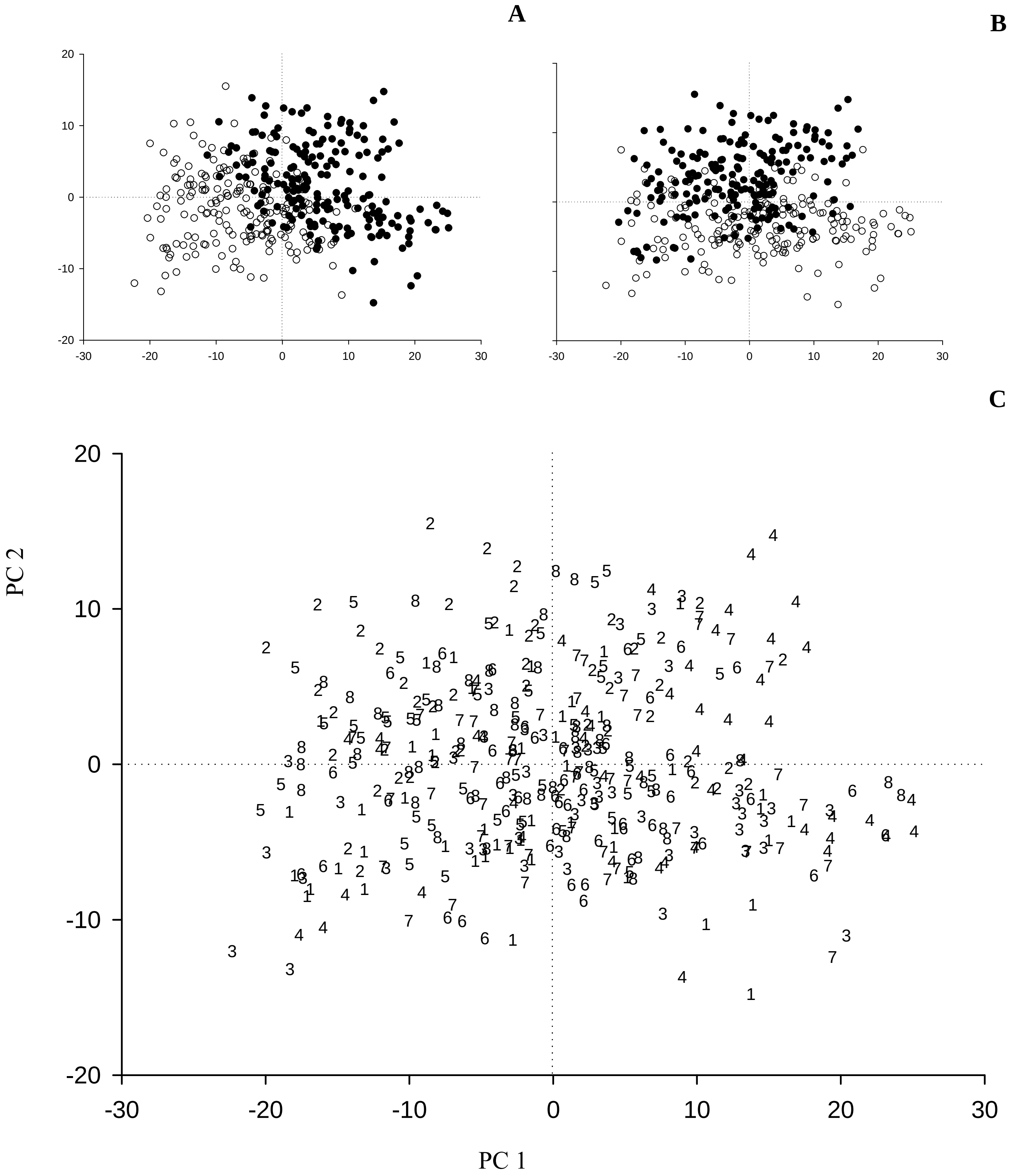


**Fig. 1** The first principal component (24.5%) plotted against the second (17.7%) for (A) herbicide-treated (filled circles) and untreated plots (open circles), (B) plots with 40% nutrient level (open circles) and 80% nutrient level (filled circles) and (C) Modena (1), Otira (2), Orthega (3), Brazil (4), Modena+Otira (5), Modena+Orthega (6), Modena+Brazil (7) and Modena+Otira+Orthega (8) with (left) and without (right) weed control. The predicted values are estimated from Scenario 1.1.

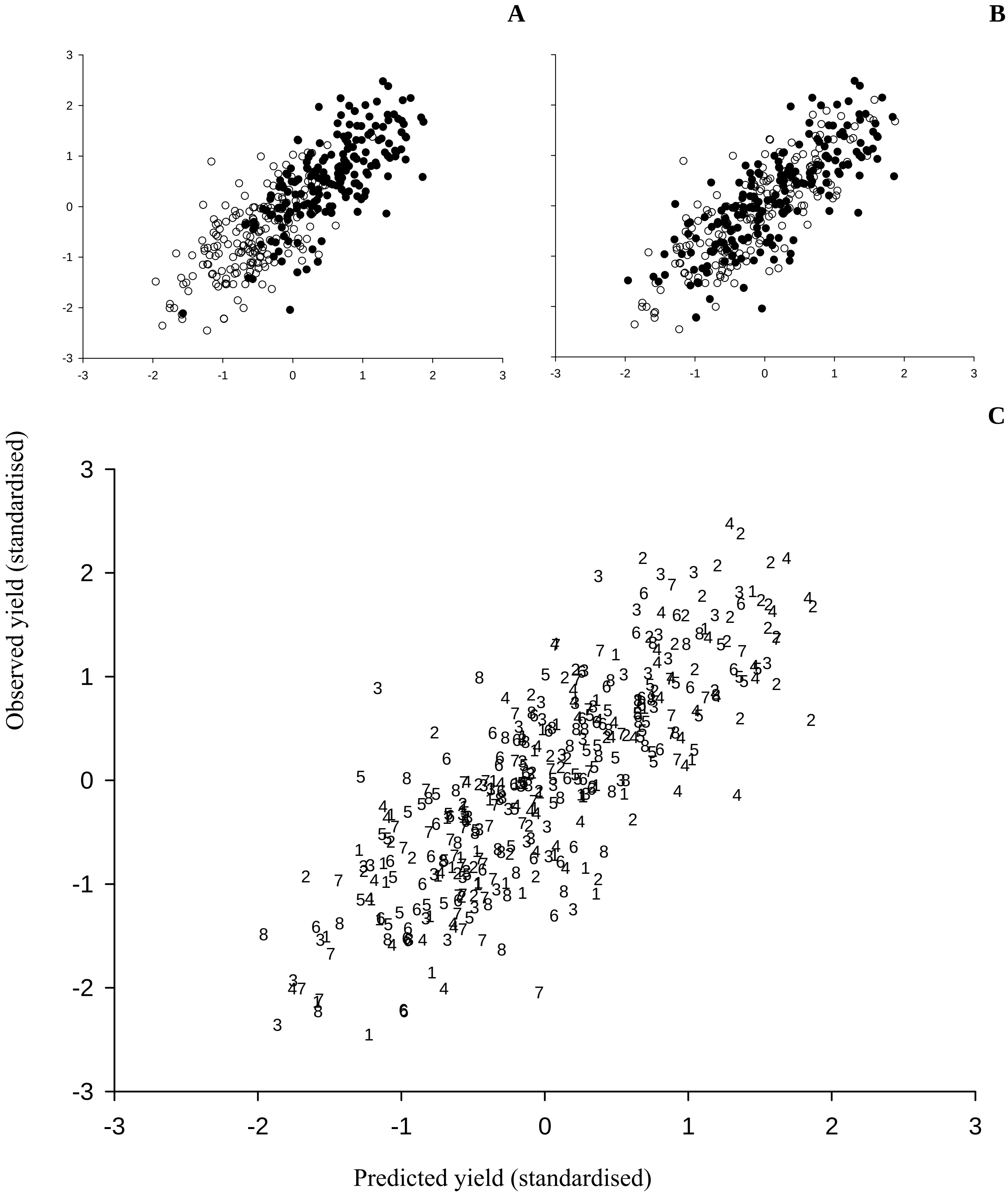


**Fig. 2** Predicted vs. observed standardised yield values for (A) herbicide-treated (filled circles) and untreated plots (open circles), (B) plots with 40% nutrient level (open circles) and 80% nutrient level (filled circles) and (C) Modena (1), Otira (2), Orthega (3), Brazil (4), Modena+Otira (5), Modena+Orthega (6), Modena+Brazil (7) and Modena+Otira+Orthega (8) with (left) and without (right) weed control. The predicted values are estimated from Scenario 1.1.

**Table 6** Results of PLS analyses of the different scenarios

| Scenario | Description | No. of PCs | Explained yield variation ($R^2$) | Root mean PRESS |
|---|---|---|---|---|
| 1.1 | All available data | 7 | 64.99 | 0.981 |
| 1.2 | Analysis 1.1 without weed density and canopy measurements | 7 | 63.77 | 1.021 |
| 1.3 | Analysis 1.1 with VIP>1.0 | 6 | 63.37 | 0.935 |
| 1.4 | Analysis 1.2 with VIP>1.0 | 7 | 65.50 | 0.947 |
| 2.1 | Date 1-5 incl. weed density | 3 | 36.52 | 1.184 |
| 2.2 | Date 1-5 excl. weed density | 2 | 22.58 | 1.272 |
| 2.3 | Analysis 2.2 with VIP>1.0 | 6 | 38.04 | 1.214 |
| 3.1 | Date 1+2 | 7 | 27.30 | 1.248 |
| 3.2 | Date 1+3 | 1 | 8.88 | 1.359 |
| 3.3 | Date 1+4 | 3 | 19.89 | 1.314 |
| 3.4 | Date 1+5 | 2 | 19.51 | 1.282 |
| **3.5** | **Date 2+3** | **7** | **31.81** | **1.301** |
| 3.6 | Date 2+4 | 3 | 25.64 | 1.275 |
| 3.7 | Date 2+5 | 3 | 26.73 | 1.235 |
| 3.8 | Date 3+4 | 1 | 13.31 | 1.324 |
| 3.9 | Date 3+5 | 1 | 17.20 | 1.289 |
| 3.10 | Date 4+5 | 3 | 23.88 | 1.285 |
| 4.1 | Date 1 | 5 | 7.29 | 1.387 |
| **4.2** | **Date 2** | **6** | **26.80** | **1.222** |
| 4.3 | Date 3 | 1 | 11.11 | 1.343 |
| 4.4 | Date 4 | 2 | 18.69 | 1.307 |
| 4.5 | Date 5 | 1 | 18.59 | 1.279 |

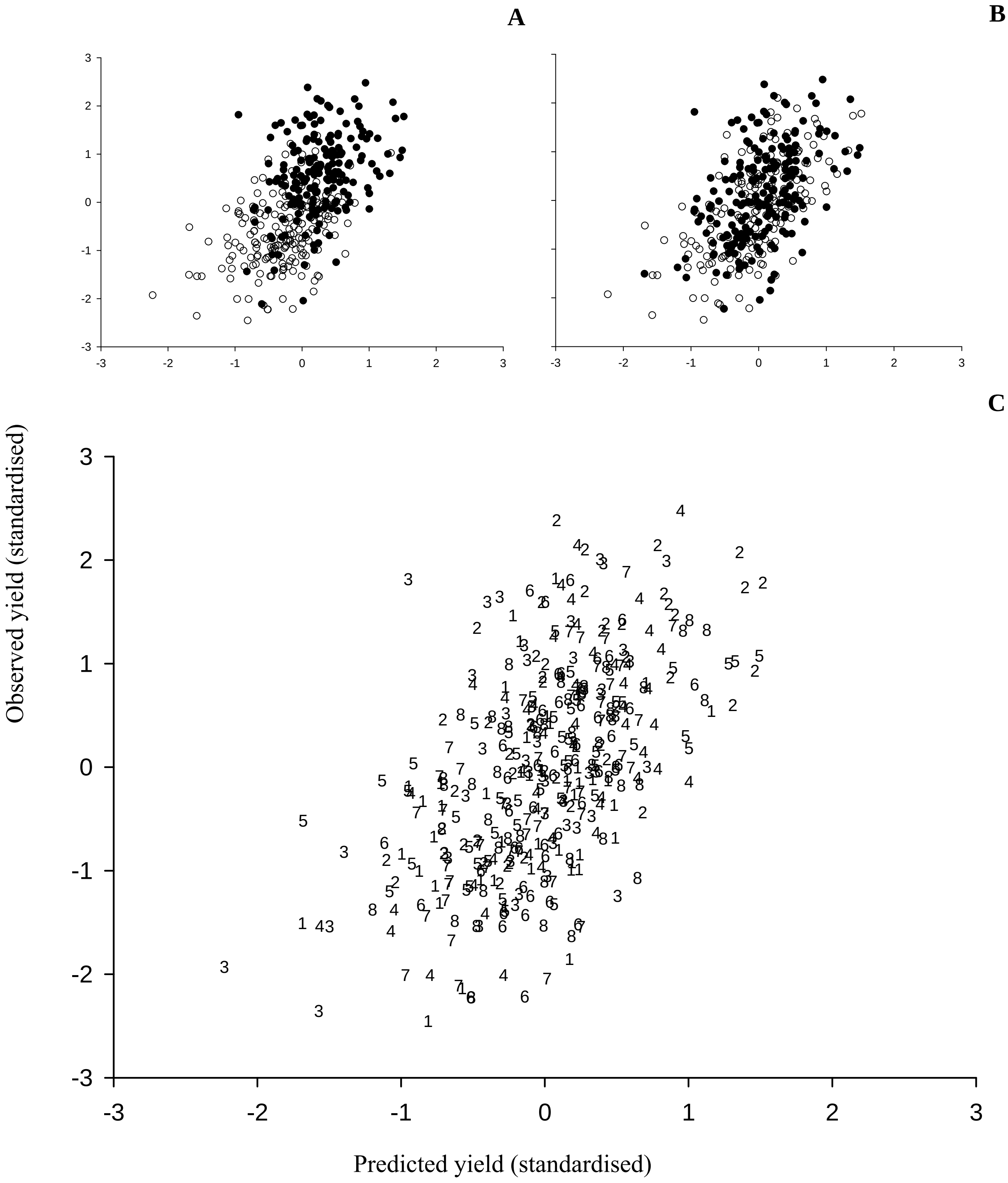


**Fig. 3** Predicted vs. observed standardised yield values for (A) herbicide-treated (filled circles) and untreated plots (open circles), (B) plots with 40% nutrient level (open circles) and 80% nutrient level (filled circles) and (C) Modena (1), Otira (2), Orthega (3), Brazil (4), Modena+Otira (5), Modena+Orthega (6), Modena+Brazil (7) and Modena+Otira+Orthega (8) with (left) and without (right) weed control. The predicted values are estimated from Scenario 3.5.

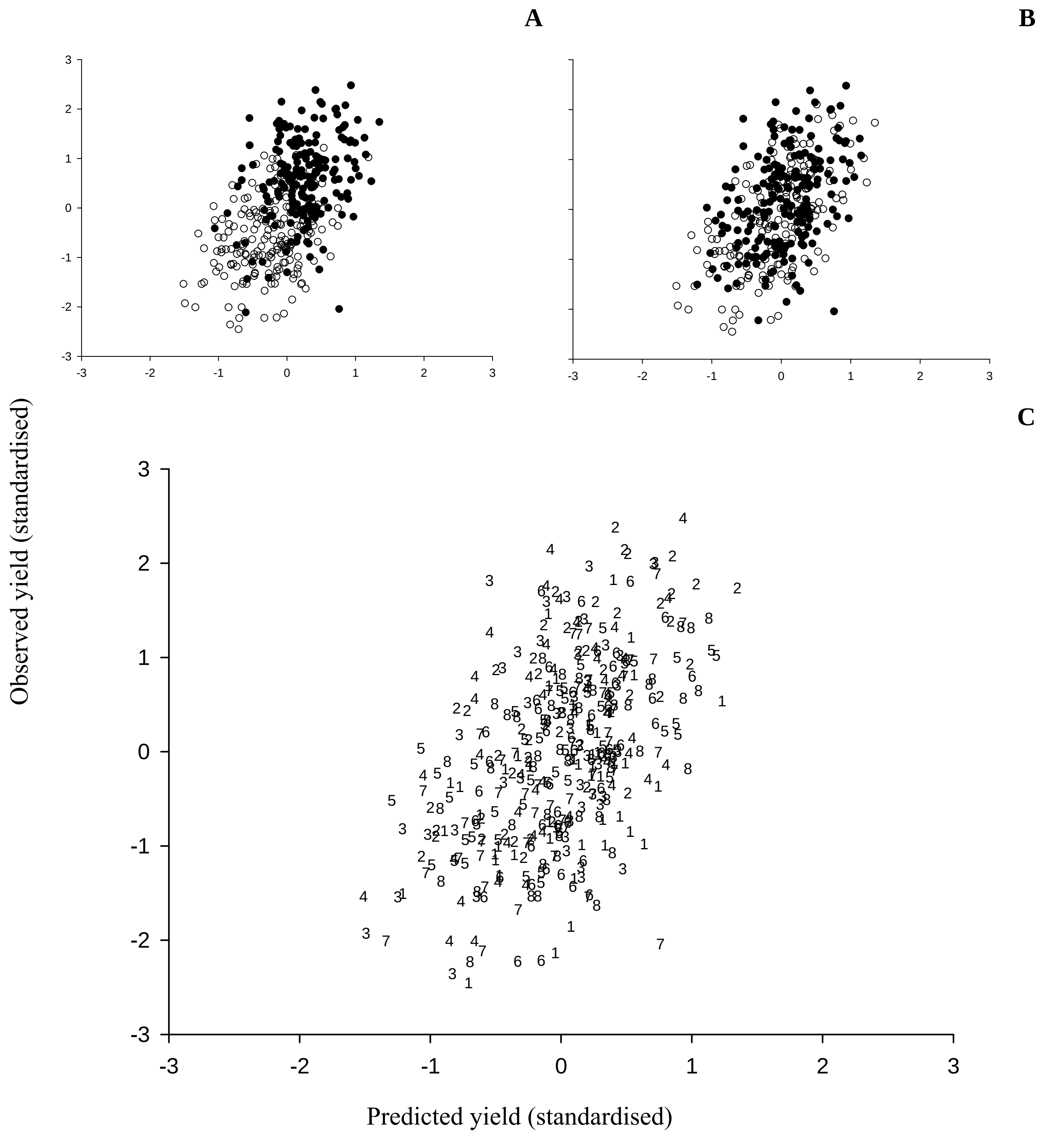


**Fig. 4** Predicted vs. observed standardised yield values for (A) herbicide-treated (filled circles) and untreated plots (open circles), (B) plots with 40% nutrient level (open circles) and 80% nutrient level (filled circles) and (C) Modena (1), Otira (2), Orthega (3), Brazil (4), Modena+Otira (5), Modena+Orthega (6), Modena+Brazil (7) and Modena+Otira+Orthega (8) with (left) and without (right) weed control. The predicted values are estimated from Scenario 4.2.

**Table 7** Predictors (X_VAR) and the corresponding VIP values and B regression coefficients from the PLS model in scenario 1.1. Predictors with VIP or B in *italics* do not contribute much to the prediction (30 lowest VIP and B values), while predictors with VIP or B in bold type are important predictors (30 highest VIP and B values). The final reflectance measurement (no. 14) together with the second vegetation cover measurement was the most important for the prediction of yield. Predictors where both VIP and B have grey background are excluded from scenarios named VIP>1.0

| X_VAR | VIP | B | X_VAR | VIP | B | X_VAR | VIP | B | X_VAR | VIP | B | X_VAR | VIP | B | X_VAR | VIP | B | X_VAR | VIP | B |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| VARIG_10 | *0.50* | 0.01 | R730_3 | 0.82 | -0.00 | R780_7 | 0.89 | *-0.00* | R710_8 | 0.93 | 0.01 | RVI_4 | 0.97 | *-0.00* | RVI_7 | 1.03 | -0.03 | REIP_13 | 1.18 | 0.02 |
| MTA_1 | *0.53* | -0.03 | R660_1 | 0.82 | 0.01 | R460_4 | 0.89 | -0.02 | R510_3 | 0.93 | -0.01 | D_CHL_AB_4 | 0.98 | *0.00* | R950_8 | 1.03 | -0.03 | RVI_14 | 1.18 | -0.03 |
| R460_9 | *0.63* | -0.01 | R660_2 | 0.83 | -0.01 | R780_9 | 0.89 | *-0.00* | R730_11 | 0.93 | -0.02 | R710_13 | 0.98 | -0.01 | RVI_11 | 1.03 | 0.00 | REIP_11 | 1.18 | 0.01 |
| TVI_1 | *0.67* | 0.01 | R460_7 | 0.83 | 0.03 | R710_4 | 0.89 | -0.02 | R760_5 | 0.93 | -0.01 | R530_4 | 0.98 | -0.03 | R760_8 | 1.04 | -0.03 | R510_13 | 1.19 | -0.03 |
| R510_8 | *0.68* | 0.01 | R780_2 | 0.83 | 0.02 | R950_7 | 0.89 | -0.01 | R760_6 | 0.93 | -0.01 | RVI_8 | 0.98 | -0.02 | VARIG_5 | 1.04 | -0.02 | COV_4 | 1.19 | **0.05** |
| D_CHL_AB_1 | *0.68* | -0.01 | R460_11 | 0.84 | -0.02 | R810_12 | 0.89 | 0.01 | R810_11 | 0.93 | -0.01 | R660_11 | 0.98 | -0.02 | VARIG_6 | 1.04 | -0.02 | D_CHL_AB_13 | 1.19 | **0.04** |
| R510_10 | *0.70* | -0.00 | R810_2 | 0.84 | 0.02 | TVI_12 | 0.89 | 0.01 | R460_12 | 0.94 | *-0.00* | R510_9 | 0.98 | -0.03 | R660_5 | 1.04 | 0.02 | REIP_10 | 1.20 | -0.00 |
| MTA_2 | *0.71* | 0.03 | R510_11 | 0.84 | -0.02 | R760_9 | 0.90 | *-0.00* | RGI_7 | 0.94 | -0.01 | DIFN_1 | 0.98 | **-0.04** | R660_6 | 1.04 | 0.02 | REIP_9 | 1.20 | 0.04 |
| R710_2 | *0.75* | *-0.00* | R810_3 | 0.84 | *-0.00* | R560_8 | 0.90 | 0.02 | R610_13 | 0.94 | -0.02 | R810_13 | 0.99 | 0.03 | R730_9 | 1.04 | -0.02 | R530_7 | 1.20 | **0.06** |
| R460_2 | *0.76* | -0.01 | D_CHL_AB_3 | 0.84 | -0.03 | R460_3 | 0.90 | -0.01 | TVI_11 | 0.94 | -0.01 | D_CHL_AB_5 | 0.99 | -0.02 | R510_7 | 1.04 | 0.03 | NDVI_12 | 1.21 | 0.00 |
| R610_8 | *0.76* | 0.01 | R660_8 | 0.84 | *0.00* | R760_10 | 0.90 | *0.00* | R760_13 | 0.94 | 0.03 | D_CHL_AB_6 | 0.99 | -0.02 | R610_5 | 1.04 | 0.01 | D_CHL_AB_14 | 1.21 | -0.02 |
| RGI_1 | *0.76* | -0.00 | R780_3 | 0.84 | -0.00 | R530_9 | 0.91 | -0.01 | R780_11 | 0.94 | -0.02 | R510_4 | 0.99 | -0.03 | R610_6 | 1.04 | 0.01 | VARIG_14 | **1.21** | **-0.07** |
| VARIG_1 | *0.77* | *-0.00* | R810_4 | 0.84 | -0.01 | TVI_9 | 0.91 | 0.01 | R530_3 | 0.94 | -0.01 | REIP_7 | 0.99 | -0.03 | NDVI_11 | 1.04 | 0.01 | R710_9 | **1.22** | **-0.04** |
| D_CHL_AB_2 | *0.77* | -0.00 | R950_3 | 0.85 | *0.00* | VARIG_9 | 0.91 | 0.01 | R610_4 | 0.94 | -0.02 | TVI_13 | 0.99 | 0.03 | D_CHL_AB_10 | 1.05 | 0.01 | R530_12 | **1.23** | -0.02 |
| R730_2 | *0.77* | 0.01 | R780_4 | 0.85 | -0.01 | R660_3 | 0.91 | *0.00* | R610_10 | 0.94 | *-0.00* | R660_7 | 0.99 | 0.02 | RVI_5 | 1.05 | -0.02 | COV_1 | **1.23** | **0.06** |
| VARIG_13 | *0.77* | -0.02 | R560_11 | 0.86 | -0.01 | RGI_11 | 0.91 | -0.00 | RVI_2 | 0.95 | 0.01 | RGI_8 | 0.99 | -0.01 | RVI_6 | 1.05 | -0.02 | R610_12 | **1.26** | -0.02 |
| R950_1 | *0.77* | *-0.00* | R530_8 | 0.86 | 0.03 | R730_5 | 0.91 | -0.00 | RVI_3 | 0.95 | -0.01 | R560_9 | 0.99 | -0.02 | R730_8 | 1.05 | -0.02 | RAP | **1.26** | -0.02 |
| R510_2 | *0.77* | -0.01 | REIP_3 | 0.86 | -0.01 | R730_6 | 0.91 | -0.00 | VARIG_4 | 0.95 | *-0.00* | D_CHL_AB_9 | 0.99 | 0.02 | NDVI_9 | 1.06 | **0.04** | R710_12 | **1.27** | -0.01 |
| R760_2 | *0.79* | 0.01 | R710_3 | 0.86 | -0.00 | R560_4 | 0.91 | -0.01 | R660_4 | 0.95 | -0.01 | NDVI_5 | 1.00 | -0.01 | RVI_13 | 1.06 | 0.03 | D_CHL_AB_12 | **1.27** | 0.02 |
| R610_2 | *0.79* | -0.01 | R560_13 | 0.86 | -0.01 | REIP_8 | 0.91 | -0.01 | R730_7 | 0.95 | **0.04** | NDVI_6 | 1.00 | -0.01 | R660_9 | 1.06 | **-0.04** | RVI_12 | **1.27** | 0.01 |
| R460_10 | *0.79* | -0.02 | R530_11 | 0.86 | -0.01 | R560_3 | 0.91 | -0.01 | TVI_2 | 0.95 | 0.01 | R710_11 | 1.00 | -0.02 | R510_5 | 1.07 | 0.01 | R660_12 | **1.28** | -0.01 |
| R460_1 | *0.79* | *0.00* | RVI_1 | 0.86 | 0.00 | R950_5 | 0.92 | *0.00* | RGI_4 | 0.95 | *0.00* | RGI_9 | 1.00 | 0.03 | R510_6 | 1.07 | 0.01 | RGI_14 | **1.28** | **-0.08** |
| R730_1 | *0.79* | 0.01 | R950_2 | 0.87 | 0.02 | R950_6 | 0.92 | *0.00* | NDVI_4 | 0.96 | *0.00* | R660_13 | 1.00 | -0.02 | D_CHL_AB_7 | 1.07 | -0.04 | NDVI_14 | **1.29** | **-0.04** |
| R810_1 | *0.79* | 0.01 | NDVI_1 | 0.87 | *0.00* | R780_10 | 0.92 | *0.00* | RVI_9 | 0.96 | 0.02 | RGI_10 | 1.00 | **0.04** | R610_7 | 1.08 | **0.04** | ROD | **1.30** | **-0.07** |
| R780_1 | *0.79* | 0.01 | R810_14 | 0.87 | -0.02 | R810_10 | 0.92 | *0.00* | NDVI_3 | 0.96 | -0.01 | R810_8 | 1.01 | -0.02 | VARIG_2 | 1.08 | 0.02 | TVI_14 | **1.30** | **-0.09** |
| R530_2 | *0.79* | 0.01 | R760_14 | 0.87 | -0.02 | TVI_4 | 0.92 | -0.01 | REIP_5 | 0.96 | -0.01 | TVI_8 | 1.01 | -0.02 | RGI_12 | 1.09 | -0.02 | COV_3 | **1.33** | **0.04** |
| DIFN_2 | *0.79* | 0.02 | R730_13 | 0.87 | 0.01 | R950_14 | 0.92 | -0.02 | REIP_6 | 0.96 | -0.01 | COV_5 | 1.01 | 0.03 | NDVI_10 | 1.09 | 0.03 | REIP_12 | **1.35** | 0.01 |
| REIP_1 | *0.79* | *-0.00* | R780_14 | 0.87 | -0.02 | R810_5 | 0.92 | -0.01 | R730_10 | 0.96 | 0.01 | COV_6 | 1.01 | 0.03 | NDVI_13 | 1.11 | 0.03 | REIP_14 | **1.35** | **-0.04** |
| R760_1 | *0.80* | 0.01 | RGI_13 | 0.88 | 0.01 | R810_6 | 0.92 | -0.01 | NDVI_2 | 0.96 | 0.01 | R660_10 | 1.01 | -0.03 | RVI_10 | 1.11 | 0.03 | R510_12 | **1.36** | -0.03 |
| R460_8 | *0.80* | 0.02 | R610_3 | 0.88 | -0.00 | REIP_4 | 0.92 | 0.02 | R780_13 | 0.96 | 0.03 | R780_8 | 1.01 | -0.02 | RGI_2 | 1.11 | 0.02 | HVI | **1.37** | -0.03 |
| R760_3 | 0.80 | -0.01 | R760_4 | 0.88 | -0.01 | TVI_7 | 0.92 | -0.01 | TVI_5 | 0.96 | -0.01 | RGI_3 | 1.01 | -0.01 | R560_5 | 1.11 | -0.01 | AND | **1.37** | -0.01 |
| R710_1 | 0.81 | 0.01 | R760_12 | 0.88 | 0.01 | R460_13 | 0.93 | -0.02 | TVI_6 | 0.96 | -0.01 | VARIG_3 | 1.02 | -0.02 | R560_6 | 1.11 | -0.01 | BLO | **1.40** | **-0.06** |
| R730_4 | 0.81 | -0.01 | R780_12 | 0.88 | 0.01 | TVI_10 | 0.93 | 0.01 | R710_7 | 0.96 | **0.05** | R710_10 | 1.02 | 0.02 | R730_12 | 1.12 | -0.00 | R460_14 | **1.43** | -0.02 |
| R560_2 | 0.81 | 0.02 | LAI_1 | 0.88 | 0.02 | R950_11 | 0.93 | -0.02 | VARIG_8 | 0.97 | -0.01 | D_CHL_AB_11 | 1.02 | 0.01 | R560_7 | 1.13 | **0.05** | R510_14 | **1.45** | 0.02 |
| R950_4 | 0.81 | -0.01 | D_CHL_AB_8 | 0.88 | -0.02 | NDVI_8 | 0.93 | *-0.00* | R610_11 | 0.97 | -0.02 | RGI_5 | 1.02 | -0.02 | R530_5 | 1.15 | -0.02 | R530_14 | **1.47** | 0.01 |
| R560_1 | 0.81 | 0.01 | R950_13 | 0.88 | 0.02 | R760_11 | 0.93 | -0.02 | REIP_2 | 0.97 | **0.04** | RGI_6 | 1.02 | -0.02 | R530_6 | 1.15 | -0.02 | R560_14 | **1.47** | 0.01 |
| R530_1 | 0.81 | 0.01 | R950_12 | 0.88 | 0.01 | TVI_3 | 0.93 | -0.01 | R710_5 | 0.97 | 0.01 | R460_5 | 1.02 | 0.03 | VARIG_12 | 1.16 | -0.02 | R710_14 | **1.47** | **0.04** |
| R610_1 | 0.81 | 0.01 | R810_9 | 0.89 | *0.00* | LAI_2 | 0.93 | **-0.04** | R710_6 | 0.97 | 0.01 | R460_6 | 1.02 | 0.03 | COV_8 | 1.17 | **0.06** | R610_14 | **1.49** | 0.03 |
| R510_1 | 0.81 | 0.01 | R950_9 | 0.89 | *-0.00* | R780_5 | 0.93 | -0.01 | R530_13 | 0.97 | -0.02 | COV_7 | 1.02 | **0.04** | R560_12 | 1.17 | -0.02 | R660_14 | **1.52** | **0.04** |
| R530_10 | 0.82 | 0.01 | R810_7 | 0.89 | -0.00 | R780_6 | 0.93 | -0.01 | NDVI_7 | 0.97 | -0.01 | R610_9 | 1.03 | -0.03 | R730_14 | 1.17 | -0.00 | HON | **1.65** | **-0.08** |
| R560_10 | 0.82 | 0.00 | R760_7 | 0.89 | *-0.00* | R950_10 | 0.93 | *-0.00* | VARIG_7 | 0.97 | -0.01 | VARIG_11 | 1.03 | -0.01 | COV_9 | 1.18 | **0.06** | COV_2 | **1.82** | **0.09** |

**Table 8** F values and levels of significance for mixed linear analysis of the residuals from the different scenarios. N=nutrient level, G=genotype, Y=year. '***' = significance at the 0.001 level, '**' = significance at the 0.01 level, '*' = significance at the 0.05 level, ns=no significance

| | Weedy | | | | | | Weed-free | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Scenario | N | G | Y | N*G | N*Y | G*Y | N | G | Y | N*G | N*Y | G*Y |
| DF | 1 | 7 | 1 | 7 | 1 | 7 | 1 | 7 | 1 | 7 | 1 | 7 |
| 1.1 | 0.26 ns | 0.55 ns | 1.11 ns | 1.06 ns | 0.25 ns | 1.52 ns | 0.16 ns | 1.92 ns | 0.93 ns | 1.19 ns | 1.03 ns | 0.84 ns |
| 1.2 | 0.62 ns | 0.61 ns | 1.69 ns | 1.44 ns | 0.73 ns | 1.44 ns | 0.20 ns | 2.12 ns | 1.40 ns | 1.20 ns | 0.80 ns | 0.82 ns |
| 1.3 | 0.12 ns | 0.80 ns | 13.50 ** | 2.16 * | 0.64 ns | 2.24 * | 4.28 * | 5.38 *** | 17.82 *** | 1.40 ns | 6.20 * | 1.60 ns |
| 1.4 | 0.24 ns | 0.99 ns | 8.26 * | 1.93 ns | 0.62 ns | 2.11 ns | 5.02 * | 5.83 *** | 18.31 *** | 1.52 ns | 5.61 * | 1.69 ns |
| 2.1 | 4.97 ns | 1.34 ns | 0.50 ns | 1.54 ns | 2.23 ns | 3.88 *** | 39.66 *** | 4.26 *** | 0.83 ns | 1.04 ns | 20.12 *** | 1.67 ns |
| 2.2 | 7.95 * | 3.51 ** | 0.52 ns | 1.50 ns | 0.31 ns | 3.32 ** | 29.51 ** | 4.57 *** | 0.68 ns | 1.36 ns | 14.84 * | 1.87 ns |
| 2.3 | 13.16 ** | 2.79 ** | 0.79 ns | 1.87 ns | 0.35 ns | 5.80 *** | 43.50 ** | 5.10 *** | 0.86 ns | 1.54 ns | 17.04 * | 2.27 * |
| 3.1 | 0.21 ns | 2.81 ** | 0.26 ns | 1.41 ns | 0.08 ns | 2.78 ** | 5.78 ns | 3.61 ** | 0.26 ns | 1.18 ns | 6.49 ns | 1.88 ns |
| 3.2 | 0.44 ns | 3.42 ** | 0.00 ns | 0.94 ns | 0.01 ns | 2.45 * | 26.00 *** | 5.13 *** | 0.00 ns | 0.93 ns | 18.04 *** | 2.14 ns |
| 3.3 | 6.74 * | 3.33 ** | 0.17 ns | 1.90 ns | 0.08 ns | 3.71 *** | 46.62 *** | 4.95 *** | 0.10 ns | 1.69 ns | 21.49 *** | 2.19 * |
| 3.4 | 10.64 * | 3.81 *** | 0.32 ns | 1.20 ns | 0.77 ns | 3.10 ** | 38.71 *** | 5.04 *** | 0.46 ns | 1.25 ns | 19.85 ** | 1.78 ns |
| 3.5 | 0.37 ns | 1.93 ns | 0.10 ns | 1.74 ns | 0.16 ns | 2.18 * | 12.72 *** | 3.12 ** | 0.08 ns | 1.74 ns | 8.55 ** | 3.27 ** |
| 3.6 | 18.88 *** | 1.84 ns | 0.10 ns | 2.14 * | 0.04 ns | 2.35 * | 50.36 *** | 3.65 ** | 0.03 ns | 1.57 ns | 14.19 *** | 2.20 * |
| 3.7 | 17.74 ** | 1.30 ns | 0.37 ns | 1.51 ns | 2.84 ns | 3.78 *** | 43.42 *** | 3.93 ** | 0.36 ns | 1.28 ns | 14.05 ** | 1.29 ns |
| 3.8 | 2.18 ns | 4.68 *** | 0.03 ns | 1.09 ns | 0.01 ns | 2.30 * | 33.48 *** | 5.48 *** | 0.04 ns | 1.27 ns | 21.97 *** | 2.31 * |
| 3.9 | 4.11 ns | 4.56 *** | 0.07 ns | 1.12 ns | 0.24 ns | 2.54 * | 42.80 *** | 5.25 *** | 0.12 ns | 1.22 ns | 21.71 *** | 2.05 ns |
| 3.10 | 10.80 * | 3.02 ** | 0.52 ns | 1.31 ns | 1.34 ns | 5.02 *** | 40.51 *** | 5.14 *** | 0.85 ns | 1.23 ns | 21.67 ** | 1.65 ns |
| 4.1 | 1.48 ns | 4.07 ** | 0.07 ns | 1.14 ns | 0.22 ns | 2.30 * | 11.71 * | 5.11 *** | 0.03 ns | 0.85 ns | 5.17 ns | 1.98 ns |
| 4.2 | 1.04 ns | 3.84 *** | 0.00 ns | 1.78 ns | 0.45 ns | 2.77 ** | 10.95 ** | 5.40 *** | 0.00 ns | 1.82 ns | 13.21 *** | 3.09 ** |
| 4.3 | 0.46 ns | 3.70 ** | 0.03 ns | 0.95 ns | 0.01 ns | 2.51 * | 26.53 *** | 5.14 *** | 0.09 ns | 1.02 ns | 19.09 *** | 2.26 * |
| 4.4 | 12.42 ** | 5.71 *** | 0.44 ns | 1.72 ns | 0.09 ns | 3.24 ** | 21.84 ** | 5.59 *** | 0.17 ns | 1.62 ns | 16.93 * | 2.21 * |
| 4.5 | 9.83 * | 3.96 *** | 0.17 ns | 1.14 ns | 0.78 ns | 2.80 ** | 44.45 *** | 5.02 *** | 0.24 ns | 1.19 ns | 21.57 ** | 1.79 ns |

## Discussion

The best prediction was gained with scenario 1.4, based on all sensor-based measurements throughout the growing season (Table 6). This model was able to explain 66% of the yield variation. This indicates that even though the dynamics through the whole growing season were measured, 34% of the variation remained unexplained. The most likely source of this unpredicted variation is the small sampling size used for the yield measurement (0.25 $m^2$). As year-to-year variation was excluded from the analysis, the predictability of the model depended on an adjustment of the yield predictions to the actual year. Furthermore, the experiments had only two treatment levels of weed control and nutrients, and therefore did not give continuous variables. However, by including the four genotypes and four mixtures of these genotypes, the strong classification effect of the results caused by the treatments was observed to be "softened" by the genotypes.

The importance ranking of the predictors showed surprisingly that the second vegetation cover estimate (COV_2) had the highest VIP and B values, indicating that this predictor was the most important in the prediction (Table 7). Behrens and Diepenbrock (2006a) also measured the vegetation cover in the early growth stages of oilseed rape (*Brassica napus* L.) by analysis of digital images and found that this measure correlated well with LAI in the early growth stages. Several studies have shown that LAI is closely related to yield (Lotz *et al.,* 1992; Kropff *et al.,* 1995; Bastiaans *et al.,* 2000). Surprisingly, the measurements from LAI-2000 (LAI, MTA and DIFN), which was conducted later in the growing season were rather low ranking with regard to VIP and B, which was in contrast to Behrens and Diepenbrock (2006b), who found that the LAI-2000 measurements correlated well with weight-related growth traits like shoot dry matter, N content and water content of oilseed rape (*Brassica napus*) and spring barley. The reason for the discrepancy could be that we conducted the LAI measurements in mixed stands of both crop and weeds.

Raun *et al.* (2001) found a significant relationship between early NDVI measurements and the yield of winter wheat. Hansen *et al.* (2003) used a three dimensional PLS method which was able to predict spring barley and winter wheat yield from four NDVI measurements in weed-free stands late in the growing season. In our study, the predictors following in importance after COV_2 were also measurements late in the growing season (measurements named "_14"; approximately one month before harvest) together with the weed density recordings (Table 7).

Pérez de Vida *et al.* (2006), however, found that in rice (*Oryza sativa* L.) early crop growth rate is important for weed suppression, while late crop growth rate (after heading) is important for yield formation. Even though we measured the total vegetation without distinguishing between crop and weed, the PLS model was able to extract some information about the competitive effect from the data. That could be caused by the relative contribution of different wavelengths in the reflectance spectrum (Vrindts *et al.,* 2002).

The optimal result from Scenario 3 (3.5) showed that only two early sensor-based measurements, which were a combination of raw measurements and extracted vegetation indices from a spectroradiometer and the estimated vegetation cover from a camera, were able to predict up to 32% of the variation in the yield measured three months later (Fig. 3). Compared with the scenario of using all available data (Fig. 2) the predictability of this scenario was only halved. By using two measurements at 10-14 days interval the growth dynamics were included in the repeated measures of light interception, thereby accounting for the important differences in time of emergence regarding weed competitiveness (Cousens *et al.,* 1987). When measured under weedy conditions in these early growth stages, the model was able to predict yield in two marked different nutrient levels in four different genotypes and individual mixtures of these genotypes without any significant divergence (Table 8). However, the model was not able to predict variety and nutrient-based yield differences under weed-free conditions without significant divergence.

In scenario 4, the second time of measurement was able to predict the yield with 27% accuracy, indicating that this time was important in the yield formation (Fig. 4). The remaining 73% unexplained variation was due to genotype, nutrient level and the interactions between these factors and year (Table 8). The genotypes had been chosen in relation to ability of weed competitiveness (Hansen *et al.*, 2007b) and not with regard to yield. One of the varieties (Modena) was a strong competitor, with a strong vegetative growth and high canopy height, but relatively low yielding. Another variety (Otira) had a more moderate vegetative growth but was very high yielding. These differences could be one of the reasons for the difficulties of the model to estimate the yields in the genotypes based on measurements before growth stage 21-22.

In conclusion, by comparing the size of the variation explained by using all available measurements through the entire growing season, an early sensor-based measurement can give reasonable estimates of the expected yield helping the farmer to optimise the use of herbicides.

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# Paper IV. Tolerance to weed harrowing in spring barley genotypes

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## Introduction

Controlling weeds in spring cereals grown under organic conditions are mostly done by post emergence weed harrowing, where spring tines of the weed harrow control weeds by uprooting and/or covering small weeds plants with soil. In situations with relatively large weed plants and relatively small crop plants, there are increased risks for crop damages by soil coverage or other mechanical damages of the crop leaves. These damages are increasing with increasing weed control intensity, and are resulting in reduced crop growth immediate after weed harrowing. There are risks that the reduced growth reduces final crop yield too. However, there is some evidence that there are varietal differences in the tolerance to weed harrowing and the tolerance is negatively correlated with competitiveness against weeds (Rasmussen *et al*., 2004).

The aim of this study was to estimate the damages by weed harrowing in four pure genotypes and three two- or one three-component mixtures of spring barley, and to analyze if there were differences in tolerance to weed harrowing between the genotypes and mixtures.

## Materials and methods

4 pure genotypes, three two-component mixtures, and one three-component mixture of the genotypes were examined for differences in tolerance to weed harrowing in field trials at Research Centre Flakkebjerg in 2003 and 2004. The field trials were designed as a split-split-split-plot-design in combination with a $\alpha$-plan (with "column-balance"). Every whole plot contained combinations of two levels of mechanical weed control (with and without a pre-emergence harrowing and one post emergence weed harrowing); two levels of pesticide treatment (with and without herbicide and fungicides) and two levels of nutrient level (40% or 80% of the recommended nutrient need).

Tolerance to weed harrowing was measured as an immediate effect (how much of the plant is covered with soil after weed harrowing), and short-term effect (growth rate after the harrowing) and a long-term effect (effect on yield). To estimate the degree of soil covering and the growth after weed harrowing, reflectance measurements were conducted immediate prior and after the post emergence weed harrowing with a CropScan MSR16R instrument (CropScan inc. Rochester MN 55906 USA). In the following 3 weeks, four measurements were conducted to measure the re-growth after the harrowing. Red Edge Inflexion Point (REIP) was estimated from the reflectance measurements and growing degree days (GDD) was used as the time-scale in the re-growth analysis.

## Results and discussion

Results from the two years field studies, shows that there are varietal differences in the tolerance to mechanical weed control in the immediate effect as well as the short term effect, however there was marked differences in the immediate and short tem effect between the two years. Regarding the long term effect of weed harrowing on yield, there was no significant differences in 2003 but in 2004, the Brazil and the three component mixture suffered significantly from weed harrowing while Modena, Otira and Modena+Orthega mixture, seems to benefit from weed harrowing, but this is not significant.

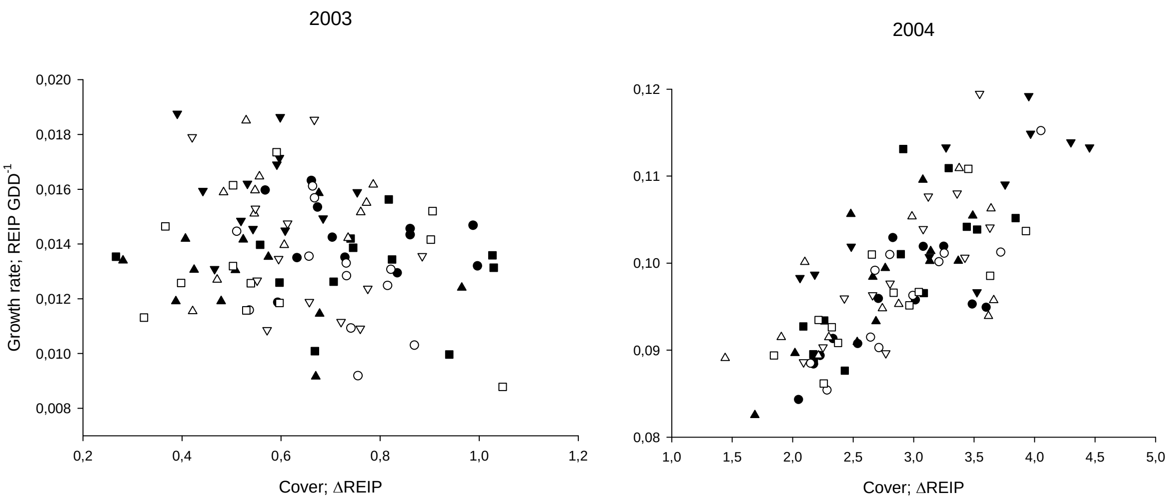


**Figure 1**. Immediate effect measured as reduction in REIP versus growth rate of Modena ( ● ), Otira ( ■ ), Orthega ( ▲ ) Brazil ( ▼ ), 50%Modena+50%Otira (O), 50% Modena + 50%Orthega (□), 50% Modena + 50% Brazil (Δ) and 33% Modena + 33% Otira + 33% Orthega (∇) in the 3-weeks period after weed harrowing in plot treated chemically. Notice different scaling on the x and y-axis

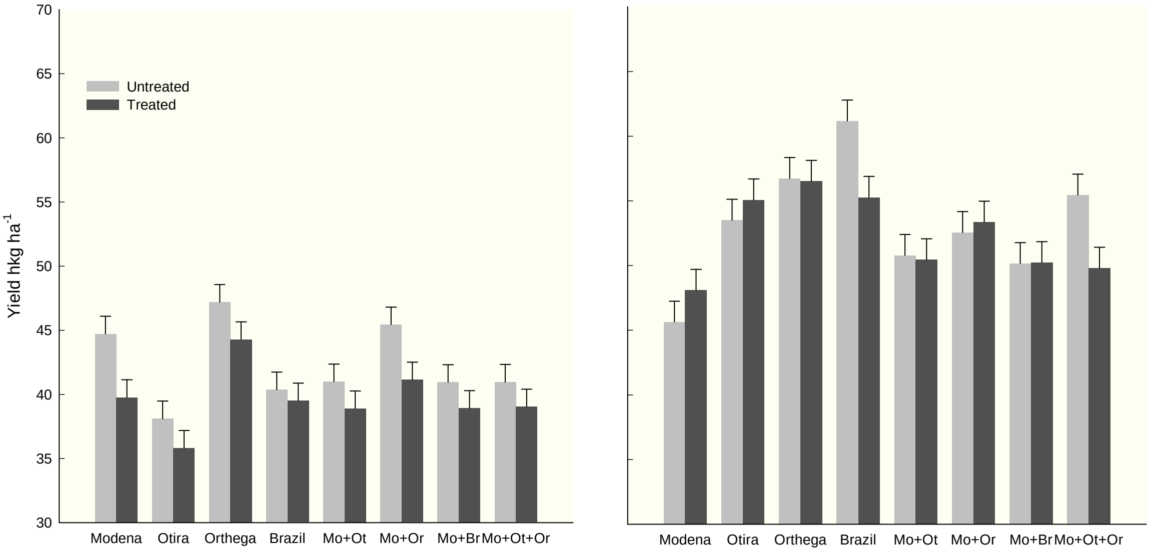


**Figure 2**.Yield of the genotypes and mixtures in 2003 and 2004 in plots treated chemically, therefore no influence from weed competition. Light grey bars indicate mean yield from two levels of slurry application in plots without weed harrowing and dark grey bars indicate plots with weed harrowing. Notice a general yield decrease in weed harrowed plots in 2003, and an yield increase in harrowed plots in 2004 with Modena and Otira and the Modena+Orthega mixture (not significant) and an decrease in Brazil (p=0.01) and the Mo+Ot+Or mixture (p=0.01).

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# Tolerance to weed harrowing in spring barley genotypes

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## Results and discussion

Results from the two years field studies, shows that there are varietal differences in the tolerance to mechanical weed control in the immediate effect as well as the short term effect, however there was marked differences in the immediate and short tem effect between the two years. Regarding the long term effect of weed harrowing on yield, there was no significant differences in 2003 but in 2004, Brazil and the three component mixture suffered significantly from weed harrowing while Modena, Otira and Modena+Orthega mixture, seems to benefit from weed harrowing, but this is not significant.

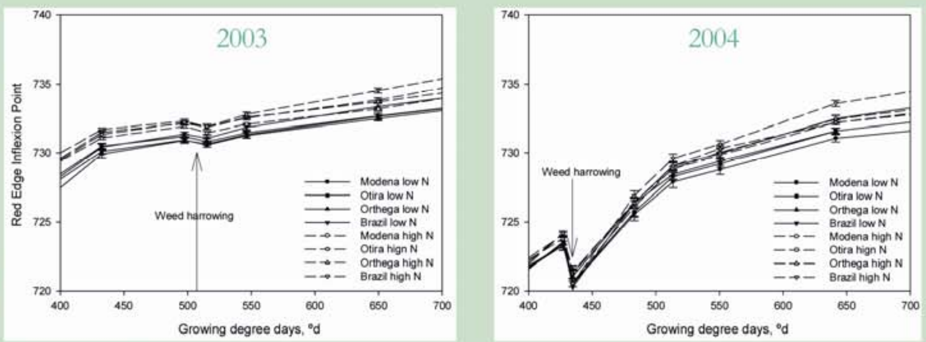


Figure 1. Red Edge Inflexion Point as a function of time (growing degree days) for four pure genotypes Modena (●), Otira (■), Orthega (▲) Brazil (▼) under low (solid lines) and high nutrient levels (dashed lines). Notice a much greater effect of weed harrowing in 2004 compared to 2003.

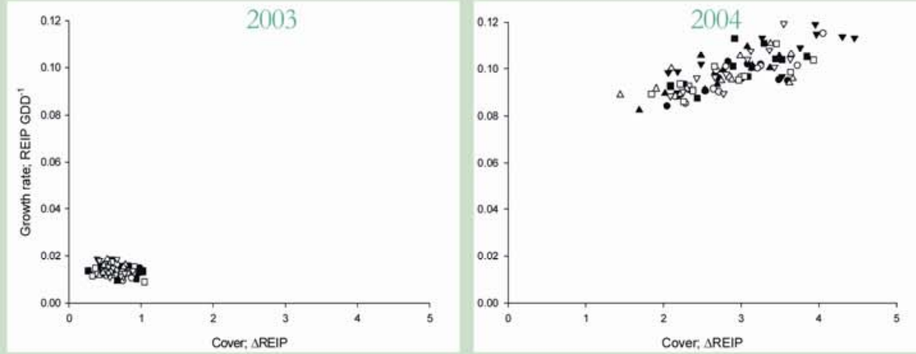


Figure 2. Immediate effect measured as reduction in REIP versus growth rate of Modena (●), Otira (■), Orthega (▲) Brazil (▼), 50%Modena+50%Otira (○), 50% Modena + 50%Orthega (□), 50% Modena + 50% Brazil (Δ) and 33% Modena + 33% Otira + 33% Orthega (∇) in the 3-weeks period after weed harrowing in plot treated chemically.

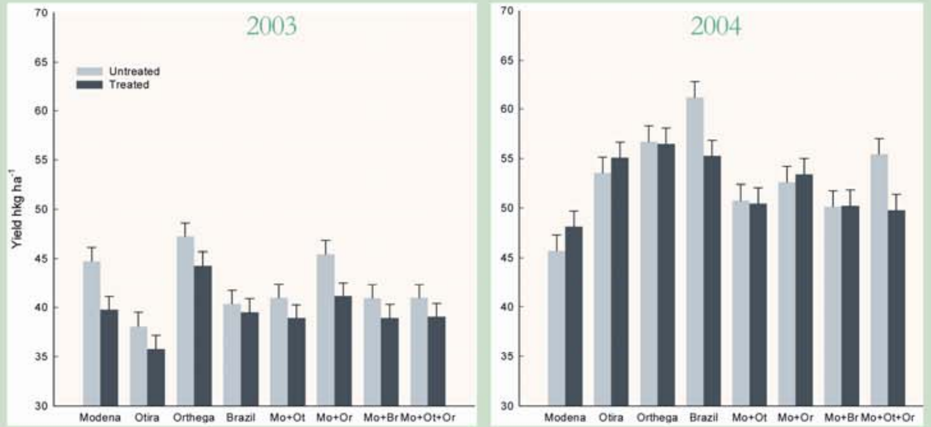


Figure 3. Yield of the genotypes and mixtures in 2003 and 2004 in plots treated chemically, therefore no influence from weed competition. Light grey bars indicate mean yield from two levels of slurry application in plots *without* weed harrowing and dark grey bars indicate plots *with* weed harrowing. Notice a general yield *decrease* in weed harrowed plots in 2003, and an yield *increase* in harrowed plots in 2004 with Modena and Otira and the Modena+Orthega mixture (not significant) and an *decrease* in Brazil (p=0.01) and the Mo+Ot+Or mixture (p=0.01).

## References

Rasmussen, *et al.* 2004. Weed Res 44, 446-452.

30APR04 | 03MAY04 | 06MAY04 | 10MAY04 | 13MAY04 | 14MAY04 | 18MAY04 | 21MAY04 | 25MAY04